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## QUARTERLY REPORT

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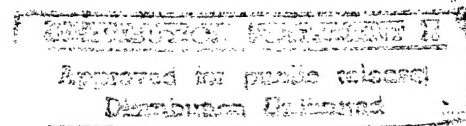
### RESEARCH ON NAVY-RELATED COMBAT CASUALTY CARE ISSUES, NAVY OPERATIONAL- RELATED INJURIES AND ILLNESSES AND APPROACHES TO ENHANCE NAVY/MARINE CORPS PERSONNEL COMBAT PERFORMANCE

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Prepared for

Naval Medical Research Institute  
Bethesda, Maryland 20814

As Required By  
Contract Number N00014-95-D-0048  
(GC-2728)



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Prepared by  
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**GEO-CENTERS, INC.**

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**QUARTERLY PROGRESS REPORT**  
**3RD QUARTER OF OPTION YEAR ONE**  
**GC-PR-2728-00**

**CONTRACT NUMBER:** N00014-95-D-0048

**REPORTING PERIOD:** June 1, 1996 - August 31, 1996

**REPORT DATE:** October 4, 1996

**RESEARCH ON NAVY-RELATED COMBAT CASUALTY CARE ISSUES,  
NAVY OPERATIONAL-RELATED INJURIES AND ILLNESSES AND  
APPROACHES TO ENHANCED NAVY/MARINE CORPS PERSONNEL  
COMBAT PERFORMANCE**

**I. INTRODUCTION**

This report summarizes the results of GEO-CENTERS' technical activities for the third quarter of the contractual option year one for the Naval Medical Research Institute (NMRI) under Contract N00014-95-D-0048, Delivery Orders 002 and 003. The delivery orders encompass a variety of scientific studies that are capable of supporting ongoing and projected programs under the cognizance of NMRI; NMRI TOX/DET-Dayton, OH; NDRI-Great Lakes, IL; the NDRI Detachment-Bethesda, MD; the National Naval Medical Center-Bethesda, MD; and the U.S. Navy Clothing and Textile Facility-Natick, MA.

The format for these periodic technical progress reports consists of four sections each listed by the location of the research. The sections are (1) Descriptions of work to be performed, (2) Objectives planned for the current reporting period, (3) Summary of work performed during current reporting period, and (4) Objectives for the next reporting period. Accumulated scientific reports, technical reports and journal articles are being provided as part of this annual technical progress report. Specifically, the research conducted by GEO-CENTERS during this quarterly reporting period has been focused on the following general scientific programs:

- A. Infectious disease threat assessment and enterics programs.
- B. Immune cell biology, wound repair and artificial blood studies.
- C. Biomedical diving programs.
- D. Personnel performance enhancement programs.
- E. Breast Care Center.
- F. Dental related diseases.
- G. Toxicological studies.
- H. U.S. Navy Clothing and Textile Facility

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## II. NMRI, Bethesda, MD

### A. INFECTIOUS DISEASE THREAT ASSESSMENT AND ENTERICS PROGRAMS

#### DESCRIPTION OF WORK TO BE PERFORMED

##### *Fernando*

- Performs research in order to develop efficient molecular assays for the detection and identification of gene sequences of orthopox viruses, which can be a warfare as well as an epidemiological threat. The research involves the study of background literature on the genomic structures and characteristics of these viruses and their current DNA detection methodologies, design of gene probes and tests that will identify and characterize orthopox genomic profiles, and evaluation and optimization of these tests for sensitivity, specificity and efficiency. These tests fall into three levels, namely, primary screening, secondary confirmatory and tertiary characterization.

##### *Ibrahim*

- Develop tests for the diagnosis and genetic characterization of Orthopox viruses.

##### *Jendrek*

- Conducts fermentations in a BL-3 suite and depending upon the organism of the fermentation may also perform some or all of the purification associated with the project.
- Creates all documentation associated with any aspect of the position, including Standard Operating Procedures, Batch Records, and any documentation required for newly installed equipment.
- Installs all new equipment related to the projects or oversees the installation by the technicians sent by the supplier.
- Assist in the Molecular Biology aspects of the position, DNA purification, Plasmid isolation, Electroporation, and other techniques are performed on a regular basis.

##### *Kerby*

- Develop diagnostic systems to detect and differentiate Orthopox viruses





*Pifat*

- Senior Scientist II
- Contractor Support for the establishment of USAMRIID's GLP capabilities

*Weeks*

- Serve as an associate of the principal investigator for a research program involving pathogenic, molecular, and biochemical analysis of bacteria and their virulence factors. Experimentation requires knowledge and proficiency of laboratory techniques and procedures for performing biochemical and immunological analyses.
- Conducts surveys of the scientific literature to develop background data on techniques and formulates approaches for the investigations, develops experimental protocols, defines the objectives and priorities of subsidiary problems and arranges the details of cooperative investigations with other organizations when necessary.
- Responsible for the general administration of the laboratory reagents, solutions, enzymes, and other materials and equipment used in conducting the studies described.
- Responsible for the cleanliness and orderliness of working areas, freezers, and refrigerators.
- Responsible for the training and orientation of all new laboratory technicians.
- Organizes and accumulates repositories of bacterial strains, plasmids, enzymes and sera with sufficient documentation of the histories of each.
- Maintains sufficient stocks of all reagents, supplies, and equipment required for a well organized molecular biology laboratory.
- Performs other duties as assigned. Immunizations are required.

**TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD**

*Fernando*

- Will optimize the secondary level DNA PCR tests for a 20 kb orthopox viral DNA amplicon. He will develop new RFLP DNA fragment labeling procedures in order to further improve the automated DNA fingerprinting analysis protocol for orthopox viruses



*Ibrahim*

- Complete the study of the 5'nuclease assay to differentiate between vaccinia and monkeypox viruses and prepare a manuscript for publication.
- Design and synthesize PCR primers and fluorogenic probes for 6 orthopox viruses' genes.
- Optimize 5' nuclease assays for at least two of these genes.
- Acquire variola DNA and conduct a blind study for evaluating the 5'nuclease assays.
- Participate in other related research activities that are important to USAMRIID's mission as necessary.

*Jendrek*

- Will continue with molecular biological aspects of the position and also start to do sequencing gels and work with the actual mutation aspect of the Protective antigen gene. The shuttle vector Scott is currently working on will then be employed in moving the mutant PA gene into various bacterial species.

*Kerby*

- Since hire date of July 22, objectives were to review literature related to molecular biology and diagnosis of Orthopox viruses, evaluate the current procedures of PCR and sequencing technology now being used at the Diagnostic Systems Division at USAMRIID.

*Pifat*

- Assist in developing pertinent SOP's and other regulatory documentation
- Assist in developing Validation plans for relevant bio-assays
- Assist in converting basic research laboratories into GLP-compliant laboratories
- Assist in selecting and establishing training courses and seminars to enhance USAMRIID's general knowledge of regulatory compliance issues.

*Weeks*

- Finish the characterization of the PLA gene of the pPst plasmid and to continue work on the new V antigen project.



## SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

### *Fernando*

- Making additional improvements on the electrophoretic method. A different type of acrylamide resin called "Long Ranger", having larger pores was demonstrated to produce, at 6-8% gel strength, better resolution of the larger RFLP fragments in the 2000-5000 bp range. Normal acrylamide at that strength caused DNA band smearing.
- Produced a 20 kb DNA amplicon of the orthopox genomes. By computer analysis a DNA stretch spanning 21,500 bp on the Vaccinia genome was selected and aligned with that of the Variola (smallpox). Then end regions having no base mis-matches (100% homology) were selected in order to design amplification primers, which was performed by using "OLIGO" software. Several sets of primers were synthesized on the automatic DNA Synthesizer.
- Evaluated these primers against the four orthopox viral DNAs. After several optimization runs, two sets of primers were established as suitable for the amplification of a 20 kb DNA segment from the pox viruses. Distinctive RFLP patterns differentiating the four viruses were also obtained with three restriction enzymes, using the ABI PRISM DNA Sequencer.
- Start developing other fluorescent labeling protocols, to produce more uniform signal intensities across the DNA fragment range. Very good preliminary results were obtained.

### *Ibrahim*

- Completed a study to develop a 5' nuclease PCR assay to differentiate between vaccinia and monkeypox viruses. Completed a manuscript.
- Designed consensus PCR primers for 7 different genes (J7R, G2R, B8R, L2R, L6R, A25R, E9L) to amplify 200-900 bp target sequences in four different species. Eight primer sets were synthesized. Two primer sets were tested for G2R, three for A25R, and three for L6R. Designed PCR primers to amplify the entire L6R, L2R and E9L genes. Designed and acquired 4 screening fluorogenic probes for testing with cowpox and monkeypox viruses.
- Analyzed the nucleotide sequences of ~500 bp fragments of the L6R genes of vaccinia, monkeypox, cowpox and camelpox viruses.



- Acquired approval of WHO to receive variola and other orthopox viruses' DNA to evaluate our 5' nuclease-PCR and RFLP assays. Contacted Dr. Joseph Esposito (CDC, Atlanta, GA) to coordinate some of our collaborative efforts.
- Tested the newly acquired ABI 7700 sequence detection system.
- Provided a briefing to the Commander, Executive Senior Scientist and Division Chief on the goals and accomplishments of the orthopoxvirus diagnostics project.
- Advised Division Chief in preparing mobile molecular diagnostics capability.

*Jendrek*

- Completed construction of the shuttle vector pSJ4 and has shown that it retains all the parts of its construction.
- Perfecting the technique for transforming *B. subtilis* with the rather large plasmid.
- Performed various fermentations in which the need for PMSF and EDTA, protease inhibitors were tested.

*Kerby*

- Organized the sequences that were available for Variola (VAR), Vaccinia (VAC), Camelpox (CML), Monkeypox (MPV), and Cowpox (CPV).
- Tested the first set of oligonucleotide primers synthesized here for the amplification of the Hemagglutinin (HA) gene, Tumor Necrosis Factor Receptor (TNFR) gene, and two RNA Polymerase (147kDa, 132kDa) genes for VAC, CML, MPV, and CPV.
- Sequenced the PCR products of the above set of primers to add to the sequence databank.

*Pifat*

- Reviewed all existing SOP's at USAMRIID and made suggestions as to how to make these documents more compliant and more user friendly. A new SOP format was devised which is now being implemented throughout the Institute. A nomenclature system was devised for all existing and future SOP's and greater than 300 necessary SOP's were identified. A number of new SOP's were written.
- Other documentation systems were implemented such as: a receipt and qualification system for Reference Materials; a buffer preparation scheme and lot numbering system; a protocol review log; communication records; equipment maintenance and repair records



- A number of Study Specific Procedures were written ,dealing with upcoming products, their quantitation and their qualification.
- A complete validation Protocol was written for the direct IgG ELISA using Anthrax PA as solid phase antigen. This is the first such document written at USAMRIID. This document will serve as a template for other a Validation Plans to be executed at USAMRIID.
- Participate in a number of planning meetings dealing with basic research laboratories to be converted to GLP-compliant laboratories. I have made suggestions concerning the types of renovations that should take place, the type of equipment that should be present and the types of documentation that should be implemented.
- Previewed two courses (Introduction to GLP's and Principles of Quality Assurance) given by "International Quality Training", with the goal of helping the instructor customize these courses for USAMRIID.

Publications/Presentations, etc.

- Participated in NCI-SAIC working group for the GMP production of recombinant PA (6/24/96, 8/26/96)
- Participated in an organizational meeting with Walter Reed on validation of assays in support of the Shigella vaccines
- Attended pre-IND meeting with the Food and Drug administration for the reduced schedule Anthrax vaccine project (7/2/96)
- Performed a site visit at Hazelton laboratories with the purpose of evaluating SOP's (7/23/96)
- Attended the annual meeting of the *Regulatory Professionals Society*, in Washington DC (September 9,10,11, 1996)
- Participated in a Battelle- USAMRIID-USAMMDA working group for the validation of the botulinum neutralization bioassay and international standards

*Weeks*

- Completion of the characterization of the PLA gene of the pPst plasmid of *Yersinia pestis* was accomplished during this quarter. Work with V antigen and its affects on human immune cells was continued during this quarter.



## GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

### *Fernando*

- Continue with experiments on other DNA labeling methods to generate more refined DNA fingerprints.

### *Ibrahim*

- Complete testing of oligonucleotide primers for all 7 genes.
- Complete testing of fluorogenic probes for 3 genes.
- Analyze entire sequences of the 7 selected genes. Re-evaluate probe design.
- Start testing variola DNA with the 5' nuclease assay.
- Complete at least one manuscript.

### *Jendrek*

- Will show if the plasmid SJ4 in *B. subtilis* makes Protective Antigen in amounts comparable to *B. anthracis* and/or greater than pMK3.
- Will start to ferment *E. coli* in order to produce F1, an immunogenic protein from *Y. pestis*.
- Will build a protocol for the fermentation and purification of F1, for which he will create batch records and SOP's.

### *Kerby*

- Design and synthesize cross-reactive PCR primers that will be used to obtain the complete sequence of the RNA Polymerase 132kDa subunit for CML, MPV, CPV, and how these compare and contrast to reported sequences of VAR and VAC

### *Pifat*

- Assist in developing pertinent SOP's and other regulatory documentation
- Assist in developing Validation plans for relevant bio-assays
- Assist in converting basic research laboratories into GLP-compliant laboratories
- Assist in selecting and establishing training courses and seminars to enhance USAMRIID's general knowledge of regulatory compliance issues.



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*Weeks*

- The objective for next quarter is to select a new research project within the scope of work in the laboratory. A literature search of on the research must be performed and at least twenty relevant articles must be read before beginning the actual work.



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## **II. NMRI, Bethesda, MD**

### **B. IMMUNE CELL BIOLOGY, WOUND REPAIR RESEARCH AND ARTIFICIAL BLOOD PROGRAM**

#### **DESCRIPTION OF WORK TO BE PERFORMED**

##### *Chavez*

- Perform basic research on the physical properties of hemoglobin and hemoglobin-based blood substitutes. Hemoglobin is the protein responsible for oxygen transport. Hemoglobin oxidation, heme stability within hemoglobin, and nitric oxide binding are the major focus area at this time.
- Provides scientific consulting on a variety of pertinent operations including analytical assay development, hemoglobin biochemistry, and protein purification and stabilization.

##### *Kidwell*

- Coordinate preparations for conferences including formatting and submitting abstracts, submitting registration fees, coordination of art work for posters, preparing travel request forms, making travel arrangements and hotel reservations, and preparing travel reimbursement forms.
- Assist in the preparation of manuscripts including editing the text, formatting the document to comply with specific journal requirements, verifying all references via MedLine, preparing the necessary number of copies and other documentation to be included, and submitting the manuscript to the journal. Track the status of all manuscripts from first draft to final publication and receipt of reprints. Respond to reprint requests for all manuscripts prepared by the Wound Repair Enhancement Program.
- Process purchase requisitions for the department in the absence of the Supply Counterpart.
- Assist in the preparation of annual reports, proposals, etc. by formatting and typing the documents, and submitting to the proper personnel.
- Assist in the preparation of animal use protocols. Track all animal use and animal per diem reports for the Wound Repair Enhancement Program.
- Work with GEO-Centers to coordinate a scientific conference. This includes reviewing site arrangements, compiling addresses for attendees, preparing letters and





invitations to attendees, preparing schedules, coordinating responses of invited speakers, and making arrangements for technical equipment.

- Provide general administrative support including writing, editing, and typing letters and memos, copying, filing, faxing, etc.
- Perform MedLine searches and obtain copies of pertinent articles.
- Assist in the preparation of Standard Operating Procedures (SOPs) for the Wound Repair Enhancement Program and maintain a central file of all SOPs for the department.
- Maintain Material Safety Data Sheet collection.

#### **TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD**

##### *Chavez*

- Completion of the pilot plant manuscript.
- Initiation of nitric oxide-hemoglobin binding experiments.
- Large scale A0 production.

##### *Kidwell*

- Finalize arrangements for the "Cell Biology of Hypoxia" conference scheduled in September 1996.
- Assist the Wound Repair Enhancement personnel in making the transition to the new organization structure, which will be occurring in the next few months, as smooth as possible.
- Continue providing support in the on-going areas such as conferences, manuscripts, animal use protocols, general administrative duties, etc.

#### **SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD**

##### *Chavez*

- We have submitted the manuscript "An Improved Process for the Production of Sterile Modified Hemoglobin Solutions" to the journal Biologicals. This journal was chosen because it emphasized biotechnology papers and it can be found on Medline.
- We have sent the manuscript "Liposome Encapsulation attenuates Hemoglobin-Induced Vasoconstriction I rabbit Arterial Segment" to the Journal of Applied



Physiology. We have made minor corrections and resubmission has already taken place.

- Two summer students, Brooke Shrader and Janis Sanders, have interned in my laboratory June 17-August 8. Their project involves a current hot topic in physiology, nitric oxide. Nitric oxide is a vasodilator, however, the exact mechanism of its contribution to the regulation of blood pressure is not known. Our study is focusing on the interaction of nitric oxide with red blood cells. Preliminary results went as expected, with the nitric oxide interaction being substantially slower with intracellular hemoglobin (red blood cells) versus extracellular hemoglobin. Further experiments are underway to determine the interaction between the red cell membrane and nitric oxide.
- The manufacturing of hemoglobin A0 on a large scale ran into several obstacles that ultimately suspended the project. Upon preparing the ion exchange columns, the CM-52 material compressed too much, causing the column pressure to exceed recommended values. After calling technical support, it was found that initial information provided to me was incorrect and the column size used was too long/narrow for the application. In addition, endotoxin levels for this column were unacceptable. A depyrogenation procedure was successfully developed and the CM-52 column was repoured at a shorter length to alleviate pressure buildups. The large scale preparation began but was unceremoniously cut off by a cracked peristaltic pump tubing used. Due to the time constraints caused by the closure of the pilot plant, the large scale hemoglobin A0 manufacturing was terminated.

Publications/Presentations, etc.

- "An Improved Process for the Production of Sterile Modified Hemoglobin solutions" F.A. Highsmith<sup>2</sup>, C.M. Driscoll<sup>2</sup>, B.C. Chung<sup>2</sup>, M.D. Chavez<sup>1</sup>, V.W. Macdonald<sup>1</sup>, J.M. Manning<sup>3</sup>, L.E. Lippert<sup>2</sup>, R.L. Berger<sup>2</sup>, and J.R. Hess<sup>2</sup>, Biologicals, submitted. <sup>1</sup>Blood Research Detachment, Walter Reed Army Institute of Research; <sup>2</sup>Bionetics Corporation, Rockville, MD; <sup>3</sup>The Rockefeller University, New York, NY.
- "Liposome Encapsulation Attenuates Hemoglobin-Induced Vasoconstriction in Rabbit Arterial Segment" A.S. Rudolph<sup>1</sup>, A. Sulpizio<sup>2</sup>, P. Hieble<sup>2</sup>, V.M. Macdonald<sup>3</sup>, M.D. Chavez<sup>3</sup>, and G.. Feuerstein<sup>2</sup>, J. Appl. Physiol., submitted. <sup>1</sup>Center for Bio/Molecular Science and Engineering, Code 6910, Naval Research Laboratory, Washington, D.C. 20375-5348; <sup>2</sup>Dept. Of Cardiovascular Pharmacology, SmithKline Beecham, King of Prussia, PA 19406; <sup>3</sup>Blood Research Detachment, Walter Reed Army Institute of Research, Washington, D.C. 20307-5100.



*Kidwell*

- Prepared and submitted five abstracts for scientific conferences.
- Coordinated preparations for investigators to attend two conferences. Began preparations for three future conferences.
- Prepared and submitted expense reports for five investigators who traveled to conferences.
- Assisted in the preparation and submission of three manuscripts. Completed revisions to one manuscript and resubmitted for publication. Began working on six additional manuscripts which are currently in progress. Tracked the status of manuscripts and filed drafts, figures and photos, and final copies.
- Processed purchase requisitions when necessary and tracked orders through fiscal, purchasing, receiving, and accounts payable.
- Assisted in the preparation and submission of a new grant proposal.
- Assisted in the preparation of one new animal use protocol and tracked per diem usage for existing protocols.
- Prepared annual reports for five projects.
- Continued to work with Dr. Nielsen and Lisa Dalton (GEO-Centers) to coordinate preparations for the "Cell Biology of Hypoxia" conference to be held in September 1996.
- Provided various administrative support to members of the Wound Repair Enhancement Program.
- Assisted in the preparation of a new SOP and maintained the SOP central file.
- Maintained the Material Safety Data Sheet collection.
- Coordinated a conference given by a visiting scientist from New York, Dr. Tanowitz.
- Responded to numerous external requests for reprints of recently published papers.
- Prepared MED 02 reports for the Scientific Director highlighting items of interest on a weekly basis.
- Utilized graphics capabilities on WordPerfect and QuattroPro to assist investigators in the preparation of presentations.

**GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD**

*Chavez*

- Continuation of the nitric oxide studies with hemoglobin.
- Further manuscript preparations.



*Kidwell*

- Complete the final arrangements for the "Cell Biology of Hypoxia" conference on September 9 and 10, 1996, attend the meeting and ensure that it runs smoothly, and transcribe the meeting.
- Continue to assist the Wound Repair Enhancement personnel in making the transition to the new organization structure, which will be occurring in the next few months, as smooth as possible.
- Continue providing support in the on-going areas such as conferences, manuscripts, animal use protocols, general administrative duties, etc.



## II. NMRI, Bethesda, MD

### C. BIOMEDICAL DIVING RESEARCH

#### DESCRIPTION OF WORK TO BE PERFORMED

##### *Cortes*

- To conduct surgeries for neurodegenerative disorders projects.
- To perform other surgeries such as cannulations and probe implants.
- To collect and analyze data from animal models.
- To assist in experiments using a hyperbaric oxygen chamber.
- To care and maintain laboratory animals in excellent condition for experiments

##### *Lee*

- Performs as a research assistant.
- Responsible for implementing and carrying out aspects of the Navy Blood Storage Project being conducted at the university of New Mexico (UNM).

##### *Shea*

##### Alzheimer Project

- To perform microdialysis experiments in the CNS of rats which have previously been lesioned at the nucleus basalis Mynert (NBM) via the drug NMDA.
- Analyze the neurotransmitters acetylcholine (ACh), norepinephrine (NE), and serotonin (5-HT), in microdialysis perfusate obtained from the above experiments.

##### Oxygen toxicity Project

- Run microdialysis experiments in the newly designed hyperbaric chambers prepared for 100% oxygen environment under deep dive conditions.

##### Dityrosine Project

- This is a new and collaborative project with the dept. of neurology at the Uniform Services University. It involves measurements of a compound which reflects oxidative stress in biological systems.



*Obowa*

- Provide technical assistance in the Diving Medicine research laboratory investigating exposure to hyperbaric oxygen (HBO) and its effects on the CNS. Prepare brain tissues for staining, section tissues using the cryostat, perform immunohistochemical staining methods on tissue sections, care for animals, perform surgical procedures on rats, and order laboratory equipment and supplies.

*Porter*

- To support in the selection of a hyperbaric CO2 analyzer for fleet submarine dry deck shelter use.
- To support analysis of fleet soda lime for possible contamination and to analyze the samples for specific dye concentrations.
- To assist with other laboratory duties as needed.

*Woolrich, McCullen*

- Coordinate air sampling and analysis of all U.S. submarines in Navy inventory.

**TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD**

*Cortes*

- To finish acquiring data for the neuroscience posters.
- To start working on the manuscript for neuroscience posters.
- To finish the microdialysis study on the effect of NMDA in the formation of free radicals in the brain.
- To train civilian staff in surgical procedures and sterile techniques.
- To start on new project involving the C-FOS protein analysis.

*Lee*

- Begin the next phase of the research project, that of in vivo human trials at the University of New Mexico approved by the FDA. This involves taking a unit of blood from volunteers, storing it under specific conditions, and reinfusing the blood to obtain 24 hour survival rates.



- Continue in vitro analysis of stored blood and begin refinement studies on blood additive solutions.

*Shea*

Alzheimer project

- To continue the experiments in the NMDA lesioned rats by increasing the number of observations at various time points post lesion.

Oxygen toxicity project

- A number of rats implanted with microdialysis probes will be run in the new hyperbaric chamber in order to monitor the effects of 100% oxygen at 3atm on the brain levels of various neurotransmitters including an amino acid profile.

Dityrosine Project

- The primary objective is to establish a specific and reliable assay for dityrosine. The system will utilize HPLC with electrochemical detection. We will also set up a number of animal and human studies to see if various stressors increase the levels of this compound in different fluid compartments.

*Obowa*

- Perform immunohistochemical staining of tissue sections for detection of c-fos.
- Expose bulbectomized rats implanted with EEG electrodes to hyperbaric oxygen, record EEG during exposure, perfuse animals, section and stain brain sections for c-fos immunohistochemistry.

*Porter*

- To continue with fleet soda lime analysis as samples come in from the manufacture.
- To continue the testing program for the candidate CO2 analyzers.

*Woolrich, McCullen*

- Prepare ORACLE database for initial data entry.
- Install atmosphere sampling kits on board initial study submarines.



- Coordinate with various Naval Commands concerning submarine atmosphere health and budget issues.
- Determine readiness of the NSMRL laboratory to conduct initial samples.

### **SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD**

#### *Cortes*

- Coordinated and supervise the use of the stereotaxic apparatus as well as surgical procedures and protocols.
- Performed cannulations, probe implants as well as other surgical procedures.
- Gathered extra data for neuroscience posters to be presented at the society for neuroscience national meeting.
- Injected animals and perfused them for the analysis of C-FOS.
- Trained civilian staff in surgical procedures such as probe implants, cannulations and cerebro-spinal fluid collection in animals as well as techniques in anesthetics and sterile procedures.

#### *Lee*

- Assisted on technical analysis of in vitro stored blood from several different experiments. Results are used to optimize the blood additive solution designed by the investigators.
- Assisted in the process of obtaining final approval by the Institutional Review Board (IRB) at UNM to conduct the Human Trial Studies.
- Began work on Human Trials. Recruited volunteer, explained the project and obtained their consent. Blood draws were conducted at the Clinical Trials Centers at UNM. Ten units of blood are currently in storage; infusions begin mid-October and run through December.
- Prepared in vitro experiment designed to assess varying levels of adenine used in the new blood additive solution. Routine in vitro diagnostics (including ATP level, vesicle production and hemolysis ) are done on a weekly basis to asses the progress of the experiment.





*Shea*

Alzheimer project

- The number of animals at various time points after NMDA lesioning has been increased.
- The analysis of neurotransmitters is on schedule and has been included in the results.

Oxygen toxicity project

- Only a few animals have been run this quarter due to technical difficulties with the microdialysis equipment during a dive. After modifications of this equipment we are now continuing with experiments. The emphasis is now on the effects of oxygen during a dive on basal transmitter release. We are doing this by potassium perfusion before and during a dive at various depths.

Dityrosine Project

- The HPLC assay for dityrosine is almost completed and we have just completed a standard curve and intra-assay variation calculations. The human urine samples were completed for this compound but there was no correlation of dityrosine with treatment. A number of other models of stress in animals has been looked at for changes in dityrosine during microdialysis but nothing has been positive as of present.

*Obowa*

- Performed animal surgeries, implanting EEG electrodes in rats to continue c-fos studies. Ordered bulbectomized rats and exposed them to hyperbaric oxygen. Stained brains of bulbectomized and control animals for c-fos immunohistochemistry.
- Performed perfusion fixation of animals and removed brain tissue. Cut brain sections for HSP and c-fos studies using cryostat. Stained tissue sections for c-fos and HSP detection using immunocytochemistry. Assisted investigators with dive chamber operation while diving rats for different projects. Ordered lab equipment and supplies.

*Porter*

- A laboratory testing plan is currently underway for the Dry Deck Shelter hyperbaric CO2 Analyzers.
- Two hyperbaric CO2 Analyzers have completed laboratory testing and are currently undergoing field testing in the fleet.



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- Two hyperbaric CO2 Analyzers are currently undergoing laboratory testing and will be sent to the fleet for field testing soon.
- One hyperbaric CO2 Analyzer has failed laboratory testing and was sent back to the manufacture for replacement / repair.
- 2 buckets of military grade soda lime were tested and approved for fleet use.
- Traveled to Little Creek Naval Base several times to work with Seal Delivery Vehicle Team 2 on the use of the hyperbaric CO2 analyzer, and the requirements of the field test plan.
- Performed other laboratory as requested.

*Woorich, McCullen*

- Installed graphical user interfaces on the ORACLE database for submarine data entry, compound entry, and CAMS data entry.
- Installed the first sampling kits aboard USS Henry M. Jackson (SSBN 730) in Bangor, WA, and aboard USS Billfish (SSN 676) in New London, CT.
- Reviewed and responded to Navy Environmental Health Center (NEHC) letter concerning "Development of Limits for Atmospheric Constituents in Nuclear Powered Submarines".
- Coordinated budget issues with OPNAV (N87).
- Coordinated readiness review of NSMRL laboratory conducted by CONTECH, Inc., of Frederick MD.

**GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD**

*Cortes*

- To finish the microdialysis trials with perfusion of NMDA to be presented at the neuroscience meeting.
- To gather more data to complete the poster to be presented at the 1996 Society for Neuroscience convention in Washington D.C.
- To assist in the experimental procedures using the implanted animals.
- To continue in the training of military as well as civilian staff of more advanced surgical procedures.
- To finish the posters for neuroscience and get ready for presentation.



*Lee*

- Assess the viability of stored red blood cell's (RBC's) in humans by in vivo 24 hr survival experiments using radioisotopes.
- Start the second half of the paired in vivo study evaluating the effect of the new additive solution alone.
- Continue the optimization of preservative solutions by storage and in vitro experiments.
- Establish RBC morphology as an in vitro diagnostic parameter.

*Shea*

Alzheimer project

- Finish the time course study for post lesioning NMDA animal studies.

Oxygen toxicity project

- Continue experiments using microdialysis in rats that will be subjected to dives involving 100% oxygen and monitor the effects from this procedure for changes in neurotransmitters and amino acids. This will include using potassium stimulation during microdialysis to determine the effects of dive upon stimulated neurotransmitter release.

Dityrosine Project

- The main focus of this project will be to refine the assay conditions and to continue to look at various stressors that will demonstrate a change in the levels of dityrosine in different biological fluids.

*Obowa*

- Finish immunohistochemical staining of the brains from bulbectomized and control animals from this study. Analyze this data to determine if brain regions affected by hyperbaric oxygen exposure are different in bulbectomized and non-bulbectomized animals. Continue HBO exposure studies based on the information from these experiments.



*Porter*

- To continue analysis of fleet soda lime for contaminants and dye concentration as needed.
- To continue testing program for dry deck shelter hyperbaric CO2 analyzers.

*Woolrich, McCullen*

- Continue refinement of ORACLE database for initial data entry.
- Interface ORACLE database with NSMRL LAN.
- Evaluate status of NSMRL laboratory.
- Evaluate success of first round of submarine atmosphere sampling.
- Coordinate with various Naval Commands concerning submarine atmosphere health and budget issues.
- Receive initial submarine atmosphere samples.



## II. NMRI, Bethesda, MD

### B. PERSONNEL PERFORMANCE ENHANCEMENT STUDIES

#### DESCRIPTION OF WORK TO BE PERFORMED

##### *Wolf*

- Provide management support to the Naval Medical Research and Development Command. Duties include reviewing medical research plans and progress reports, recommending laboratory guidance, evaluating research proposals, drafting periodic and ad hoc management reports and developing presentation materials.

##### *McCowin*

- Provide management support to the Special Operations Forces Medical Technology Development Program at the Naval Medical Research and Development Command. Duties include reviewing and evaluating medical research proposals, reviewing incremental reports and comparing them with the approved research plans, and drafting periodic and *ad hoc* management reports, developing draft presentation materials and managing financial budget. The scope of research includes all topics within the Special Operations Forces Medical Technology Development Project. This includes investigations relevant to the treatment of disease, trauma, effects of environmental extremes and treatment for medical support of Special Operations Forces operations. In addition, from time to time, collect, process and report findings on critical issues which are directly related to other urgent military medical research issues within the purview of the Special Operations Forces Medical technology Development Program

#### TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

##### *Wolf*

- Continue to identify those areas where Geo-Centers, Inc may assist NMRDC in the transition to whatever its future will be (it should be pointed out that this is difficult in that we do not know from day to day what the future is). Facilitate the production of reports for the Director of Research and Development.



*McCowin*

- Collect monthly obligation and expenditure reports from principal investigators.
- Reviewed and distributed 2nd incremental progress reports.
- Draft and submitted Broad agency Announcement (BAA) to ONR for incorporations into the Commerce Business Daily (CBD).
- Send request for pre proposal package to FY97 New Start Project.
- Send request for proposal package to project manager of FY97 new start tasking statements.
- Submit FY95 and FY96 obligations and expenditure report to Special Operation Acquisitions Center (SOAC) for Execution Review Conference.
- Submit monthly FY95 and FY96 unobligated funds report to SOAC.
- Prepare draft Memorandum of Agreement (MOA) between US Special Operations Command (USSOCOM) and The Naval Medical Research and Development Command (NMRDC).
- Attend Biomedical Initiative Steering Committee (BISC) meeting Sept 96.
- Evaluate work unit file of principal investigators for funding and deliverable status.
- Submit final baseline agreements for all SOCOM funded projects to be signed by Program Manager, BISC Chairman, and SOAC Deputy of Acquisition.
- Prepare new baselines for FY97 new start SOCOM funded projects.
- Prepare FY97 funds for distribution in conjunction with BISC chairman.
- Prepare and submit FY97 funding requirements for Med-Tech program for the Program Objective Memorandum (POM).
- Prepare and present FY98 budget briefing to SOAC Deputy of Acquisition and OSD/OMB.
- Brief Program Manager, Comptroller, and Commanding Officer on the status of the new Program Base Accounting system (PBAS).
- Attend PBAS training course.
- Install PBAS training and TELNET capabilities on the Comptrollers computer and Program Manager computer.



## **SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD**

### *Wolf*

- Continued to build a data base for investigator accomplishments. It is the goal of this data base to allow the Director of Research and Development to readily pull together all the significant actions which have been accomplished by our funded investigators. It is intended, also, that this data base will feed into the major work-unit-tracking data base the command has commissioned from a separate contract. (As an aside, I've been selected as a "beta tester" for this command information management system, a major portion of which is the work-tracking-system ... and have identified four medium-sized program deficiencies, to date.)
- Assisted all Program Managers in preparing various presentation charts, vu-graphs and slide shows for use at the time of the visit by N931, RADM Phillips to NMRDC. The visit was in mid-July, and some 40 vu-graphs and slide shows were developed for the briefing.
- Was able to convince Office of Naval Research that modem connection to the INRIS program was imperative, both for NMRDC and its own Program Officers. Now that modem connection is available, completed one "proposal action" and two PR actions, WITHOUT having to commute to Ballston Towers (saves command funds and time).
- Assisted the command in preparation for the August Wargame Process "VANGUARD 96", the FIRST medical wargame. As best I know, three of my suggestions for "play topics" were used in the gaming. I also prepared five briefing books for distribution to Flag Officers.
- Although there has been no final closure, I've completed 95% of the administrative work for the \$4.5M Coastal Cancer Research Program to be funded at Medical University of South Carolina. This included the proposal review and update, the INRIS "proposal action" and the INRIS "procurement request". I've also had to track the funding closely as Army and Navy comptrollers have had difficulty in initiating the correct paper work.

### *McCowin*

- Work from Technical objectives section was performed during this reporting period.



## GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

### *Wolf*

- Continue to identify those areas where Geo-Centers, Inc may assist NMRDC in the transition to whatever its future will be. The continuing volatility of this situation makes it difficult on all hands in the planning of such a transition, but the simulations are interesting, that is for certain.

### *McCowin*

- Attend Special Operations Medical Association (SOMA) Meeting and BISC Meeting in Dec 96
- Collect and Evaluate 3rd incremental progress reports.
- Collect monthly obligation and expenditure reports from principal investigators.
- submit monthly obligation and expenditure reports to SOAC.
- Prepare and submit final draft of the MOA between US Special Operations Command (USSOCOM) and the Naval Medical Research and Development Command (NMRDC).
- Provide input for the reversion of the USSOCOM Project Reference Book.
- Provide input for the revision of the USSOCOM Technology Execution Plan.
- Become familiar with PBAS and use to distributed FY97 funding.
- Prepare files of all USSOCOM projects for the incorporation into new 3-D Uninex-based computer program system.





## II. NMRI, Bethesda, MD

### E. BREAST CARE CENTER

#### DESCRIPTION OF WORK TO BE PERFORMED

*Patient Service Representatives*  
*Grimes, Jenkins, Lozoya, Williams*

- Develop a system for processing and interviewing patients, incorporating standard patient registration procedures. Maintain uniform policy for check-in/check-out procedures.
- Devise a system for completion and collection of third party insurance forms on each patient.
- Perfect receiving patients and incoming telephone calls/inquiries, determine priorities and refer to proper person/department.
- Ensure that all incomplete patient records and third party forms are corrected or returned to proper staff for completion/correction.
- Set up records and filing system for paperwork associated with each patient record. Ensure that all documents processed are in accordance with department standards and that all forms are in designated order in the patient records. Label files for permanent shadow files.
- Assist with establishment of standard operating procedures.
- Orient new support team members and clinical team staff to office routine.
- Devise a system of notifying all no-shows, record information in shadow file and initial.
- Call/notify all physicians the day before they are scheduled for clinic; let them know approximately how many patients they will see.
- Print Composite Health Care System (CHCS) daily schedule and end of day reports. Check end of day report for accuracy.

*Balintona*

- Responsibilities include being responsible for addressing the psychosocial status, mental status, patient concerns, and the impact of diagnosis on family relationships of breast cancer patients.



- Assess newly diagnosed breast cancer patients and provide them with social work educational materials. The assessment includes a screenign for depression, adjustment, patient social history and support systems available to patient.
- Facilitation of the Stage I & Stage II Breast Cancer Survivors' Group
- Facilitate the Advanced Breast Cancer Support Group
- Facilitate the Spouse's of Breast Cancer Patient's Support Group
- Collect and analyze research data on the Adjustment and Social Support in Male Spouse's of Breast Cancer Patients.
- Liaison with the National Naval Medical Center Social Work Staff . Attend all social work staff meetings to coordinate communication and colleague interaction.
- Coordinate individual, family, group and marital psychotherapy based on Social Work assessment and clinical intervention needed

*Blankenship*

- Provide care for patients with both malignant and benign diseases of the breast, including: initial evaluation, definitive medical or surgical therapy, and long term follow-up.
- Educate medical students, residents, nurses, and other physicians in the diagnosis and treatment of malignant and benign diseases of the breast.

*Durand*

- Acts as a liaison between the patient and the family and all other health care providers, intervening at key points (and or when significant problems occurs) for individual patient. Addresses and resolves issues that have a negative impact , creating opportunities and systems to enhance positive outcomes.
- Performs on site visits with patients in various clinical areas.(i.e. Radiology Oncology (Rad. Onc.) Medical Oncology (Med. Onc.) and Post-Op areas.
- Initiates and contributes via multidisciplinary team approach modifications or changes in caregiver practice patterns to maximize quality patient care and resource utilization.
- Assists in the development and implementation of the Task Manager program with Digital Equipment Corporation.
- Checks daily for outstanding biopsy results.
- Page and inform physician of biopsy results if positive.
- Make follow-up phone calls to post operative patients to check on their well-being, this can be weekly, monthly, and every 3 months.



- Schedule follow-up appointments to Rad. Onc, Med.Onc, Physical Therapy, Nuclear Medicine, and C.T. Scan.
- Verify consults to clinical areas for breast cancer patients with follow-up phone call.
- Prepare information data form for Tumor Board presentation of positive cancer patients.
- Follow-up visit to clinical areas with patients after surgery.
- Verify surgical dates via surgical clinic and OR schedules.
- Perform pre-op teaching specific for nurse case manager with patient and family members.
- Acts as support system for patient and family , in conjunction with the Social Worker and Nurse Educator for newly diagnosed cancer patients.
- Acts as supervisor for 2 Registered Nurses preparing evaluations and preparing time sheets exclusively.
- Instructs post-op mastectomy patients on breast prosthesis, and issues dealing with the appointments for fitting.
- Initiate order forms with prescription for the patient's acquisition of breast prosthesis.
- Acts as liaison between prosthetic company and patient/clinic.
- Hand delivers consults to various clinical areas.
- Establish a method for efficiently linking the Case Manager with the patients within the identified ulations population as early on the trajectory as possible.
- Monitor and evaluate the patient's status vis-à-vis the plan and the anticipated intermediate goals and outcomes in a Timely Way.
- Negotiate activities needed to keep or return the patient to the anticipated path.
- Negotiate system changes to address patterns identified in aggregate variances.

*Richman (Fields)*

- Perform technical services including mammograms.
- Assisting in biopsies and ultrasounds.
- Perform quality control.

*Higgins*

- To begin to define and develop the roles and responsibilities of the Research Nurse position
- Develop an orientation schedule for the Research Nurse position
- Coordinate with the Cancer Genetics Nurse from NCI an agenda for orientation with nurse researchers from Bethesda, NCI and NIH



- Literature search on current trends in the area of nursing research
- Literature review of BRCA1 and BRCA2 testing and patient education
- Data collection
- Patient education relating to protocols
- Liaison between BCC and other governmental/research institutions
- Coordinate research proposal/grant development
- Discussion and planning of data base development within the BCC
- Becoming familiar with the tracking system of patients involved with NSABP protocols
- Meeting with the Clinical Nurse Researcher and medical staff in Hem/Onc
- To start to utilize Care Manager to identify trends of care in the BCC
- Investigating possible journal subscriptions relevant to the Research Nurse position
- Keeping the BCC staff abreast of research issues relevant to patient care and staff development
- Attending seminars/conferences for staff and professional development
- Co-investigator on a multidisciplinary team of researchers involved with BRCA1 and BRCA2 testing in the BCC
- Participation and case study presentation at BCC staff meetings and multidisciplinary meetings

*Lopez*

- Develop and integrate a breast care educational program for female Department of Defense beneficiaries and their support persons.
- Educational program to include all breast care issue with an emphasis on early detection of breast cancer.
- Provide pre-operative teaching and educate patients regarding breast cancer and treatment options.
- Being available as an information resource person for the patient and their support person.
- Plan staff development programs and maintain BCC staff development records.
- Act as relief Ambulatory Care Nurse under the direction of the nurse manager.
- BCC designated safety representative, responsible for safety manuals, monthly safety meetings and BCC safety issues.



*McIntyre*

- Support a research program which focuses on breast cancer.
- Liaison between the Radiology Department-Mammography Section, the Breast Care Center (BCC), and other hospital departments.
- Performs nursing duties.
- Performs managerial duties

*Portee*

- Coordinates patient flow activities.
- Performs professional nursing assessments.
- Teaches breast self-examination.
- Prepares patient charts with appropriate medical, lab, and x-ray reports.
- Assists physicians with all procedures such as FNA or cyst aspirations.
- Provides physical and emotional support to patients during appointments.
- Collaborates with a multi disciplinary staff concerning patient needs and identifies patients who may benefit from services such as social service, physical therapy, or nurse case management.
- Responsible for preparing all clinical areas for patients and securing clinical areas at the end of the day.
- Processes linen and hazardous wastes within the BCC.
- Maintains supplies at par level and records supplies needed.

*Prindle*

- Coordinates patient flow activities
- Acts as relief clinical nurse manager in the absence of nurse manager
- Collaborates with physicians concerning unscheduled patient appointments
- Performs professional nursing assessments
- Teaches breast self examination and pre and post biopsy education
- Triage patient phone calls and consults with physicians as needed
- Prepares patient charts with appropriate medical, lab, and x-ray reports
- Responsible for entering physician orders into computer
- Assists physicians with all procedures such as FNA or cyst aspirations
- Provides physical and emotional support to patients during their appointment



- Collaborates with a multidisciplinary staff concerning patient needs and identifies patients who may benefit from services such as social service, physical therapy, or nurse case management
- Management of clinical supply needs
- Supervising clinical nursing staff: 1 RN and 1 LPN
- Team Leader for clinical practice

*Snee*

- Case manages new breast cancer patients
- Utilizes the "Care Manager" software to follow a patient through her care and to document her treatment
- Helps to educate newly diagnosed breast cancer patients about disease, treatment, and follow up care
- Provides educational materials to patients and families
- Coordinates and plans appointments for multidisciplinary care in hospital, including, but not limited to hematology/oncology, radiation/oncology, plastic surgery, physical therapy, and social services
- Teaches patients about prosthetics and assists patient in preparing appropriate forms necessary to obtain prosthetic
- Provides emotional support to women and their families who are facing cancer treatment through verbal and nonverbal communication
- Provides support, comfort, and education to the patient through the use of pre and post op phone calls and by visiting the patient while they are an inpatient.
- Ensures that patients are receiving adequate follow up care
- Prepares forms necessary to present patients to weekly tumor board meeting and ensures that all new breast cancer patients are presented
- Tracks breast biopsies and notifies doctor of any malignant pathology reports and ensures that patient is scheduled for appointment with physician
- Assists as needed in clinic as either ambulatory care nurse or nurse educator

*Taylor*

- Manage and maintain the conference room schedule and database.
- Assist with research projects as well as devise a database to store the data.
- Perform desktop publishing, word processing, and other secretarial/clerical functions.
- Provide end user support.
- Write, edit, update correspondence and standard operating procedures



- Maintain electronic filing system for thank you letters and patient no shows and follow up care.
- Order supplies for various departments within the center.
- Manage and maintain the procurement process and database.
- Generate reports relative to supply issues
- Point of contact for procurement and the conference room
- Assist Patient Service Representatives.
- Attend meetings as assigned.
- Ensure all mail is picked up and delivered daily.
- Organize and label supplies.
- Monitor supplies on hand

*Vaughn*

- Medical filing for the Radiology department and the Breast Care Center.
- Enter CHCS orders for comparison mammograms.
- Track films.
- Handle mail and telephone correspondence regarding radiology films.
- Pull and file mammograms.
- Make copies of mammogram films for physicians.

*Wallace*

- Coordinate administrative activities of the Breast Care Center (BCC). In absence of Administrator and Nurse Manager, act as primary administrator of the BCC.
- Manage patient/physician schedule templates in the Composite Health Care System (CHCS).
- Collect and report all workload.
- Manage budget.
- Work with administrative team to develop Strategic Plan, Information Systems Plan and Marketing Plan for guiding future clinic operations.
- Oversee implementation of the Ambulatory Data System (ADS) for the BCC.
- Oversee procurement ordering process.
- Assist Contract Management Department with maintaining accurate and complete files on BCC employees.
- Assist in preparation for VIP tours and briefings.



## TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

### *Patient Service Representatives Grimes, Jenkins, Lozoya, Williams*

- Streamline and organize front-desk procedures.
- Retrieve and ensure completion of third party insurance forms
- Improve routing and response to incoming telephone calls/inquires
- Use standard registration procedures requiring plastic green card for imprinting all forms pertinent to each patient.
- Provide roster for pulling mammogram films 1-2 days prior to the scheduled visit.
- Coordinate policies for scheduling appointments/procedures for patients calling/walk-ins/consults/cards.
- Streamline physician schedule notification process.
- Refine CHCS daily schedule and end of day reporting.
- Ensure pick-up and delivery of mail in a timely manner.

### *Balintona*

- Started a new group in the Breast Care Center that will meet once a month. The group will include education about different aspects of breast cancer by military health care providers followed by a support group run by the BCC social worker. This group has been developed and will begin in October.
- Organized and coordinated the American Cancer Society Look Good...Feel Better program into the Breast Care Center. This program is geared towards enhancing women's self-esteem and body image following a cancer diagnosis which enhances patient psychosocial/emotional adjustment. The Breast Care Center patients have the opportunity to attend this program within our center on the first Monday of each month.
- Developed Social Work integration with the Breast Care Center Care Manager computer program. This included formation of a social work critical pathway, patient integration and chart documentation according to the Care Manager format.
- Provided individual psychotherapy to patients experiencing significant emotional distress following diagnosis. Also provided on-going therapy to patients who have experienced specific types of concerns at the completion of treatment including sexuality/intimacy issues, fear of reoccurrence and family support post treatment.
- Addressed the psychosocial status and mental status of individual patients in the Breast Care Center





*Blankenship*

- Maintain the high standard of care that has been established at the Breast Care Center and provide comprehensive care to patients with diseases of the breast.

*Durand*

- Continue to improve Task Manager through ongoing collaboration with Digital Equipment Corporation.
- To continue to develop through multidisciplinary collaboration, Standards of Procedures (S.O.P.) for the Nurse Case Manager's position..
- Continue to enter in" Care Manager" program all newly diagnosed Breast Cancer Patients, and biopsies.
- Refine techniques for capturing statistical data that would impact future studies of the Breast Care Center.
- Attend seminars/conferences regarding Case Management for CEU's and improvement of my position as Case Manager.

*Richman (Fields)*

- Perform various studies within the department thereby increasing knowledge and experience.
- Broaden understanding of the BCC's procedures and personnel. Expand relationship with BCC.
- Will take full advantage of any educational opportunities which may arise as time and schedule permits.
- Continue to increase knowledge of mammography and breast diseases using the doctors as teachers.

*Higgins*

- Investigative tool to assess variables for nursing research within the BCC
- Organization of work area and files to begin Research Nurse position
- Develop an orientation schedule and agenda for the Research Nurse position
- Further develop computer skills utilizing Windows -NT software
- Meet with Digital to install Care Manager software on my PC and initiate usage
- Patient education guidelines relating to research participation and information



- Utilize "Guidelines for Research Proposal" program to start to develop protocols for BCC
- Begin to investigate available statistical software programs

*Lopez*

- Will continue to provide patient education.
- Develop a video library reference guide for patients.
- Implement discharge instruction brochure after approval.
- Continue to act as relief ambulatory care nurse.
- Continue to develop array of patient educational materials.
- Continue staff development and safety representative responsibilities.

*McIntyre*

- Assist the Radiologists/staff with stereotactic and ultrasound guided breast biopsy procedures.
- Perform assessments on all stereotactic/ultrasound biopsy patients and provide these patients with post breast biopsy teaching instructions.
- Assist with continued development between BCC and Radiology department.
- Design Breast Core Biopsy brochure for patients

*Portee*

- Continue development in the role of the ambulatory care nurse.
- Inventory needles and syringes.
- Continue development of computer skills, especially the use of hospital's CHCS system.
- Continue ongoing evaluation and revision of nursing assessment tool.
- Continue to gain further knowledge and education in breast cancer and it's treatment.

*Prindle*

- Continues development in the role of the ambulatory care nurse
- Continues development of computer skills, especially the use of the hospital system called CHCS
- Ongoing evaluation and revision of nursing assessment tool
- Continue to gain further knowledge and education in breast cancer and it's treatment



- Ongoing development and assessment of nursing protocols for telephone triage
- Continue working with Patients Service Representatives to achieve a fluid transition between PSR/Patient/Nurse (Developing Algorithm format)
- Develop role as Team Leader for clinical practice
- Participate in staff interviews for clinical nursing positions
- Supervise/Train new staff nurses in clinic procedures
- Schedule new staff into computer training courses
- Develop new appointment template system to better utilize patient flow
- Develop new chart system with management staff to accommodate multiple disciplines : rad/onc and hem/onc
- Develop new algorithm format with Digital for clinical process

*Snee*

- Ongoing development in the role of the nurse case manager
- Ongoing development of computer skills, especially the use of hospital's system called CHCS and the nurse case manager software called "The Care Manager"
- Implement processes that will enable appropriate follow up care for breast cancer patients
- Continues to revise and perfect methods to discuss cancer diagnosis with patients
- Continues to gain further knowledge and education in breast cancer and it's treatment
- Ongoing development of organizational skills to manage multiple patients and their individual needs

*Taylor*

- Learn the objective and processes of the breast care center
- Streamline and organize office procedures to promote a work smarter environment.
- Ensure a timely distribution/pick up of inter-office mail, etc.

*Vaughn*

- Alphabetize the main mammography file system.
- Systematic checking for quality improvement.
- Improve report filing to allow for more efficient operations.
- Being readily available for assistance to CO-workers, the BCC staff, physicians and patients requiring assistance with mammography films.



*Wallace*

- Coordinate administrative activities of the BCC.
- Assist in preparation of Statements of Work for obligation of future funding.
- Manage schedule templates for BCC attending physicians.
- Collect and report all monthly workload.
- Develop system for expenditure tracking, and research patient level accounting system.
- Research alternative method for patient charting to reduce amount of paper files.
- Develop Strategic Plan and Marketing Plan, Information Systems Plan.
- Collect information on appointment accessibility and track patient access to determine potential bottlenecks for appointment shortages (i.e. determine reason for lack of availability).
- Develop curriculum for administrative intern.
- Oversee implementation of ADS for the BCC. Attend weekly meetings of the Information Management Quality Management Board to keep up-to-date on all information systems issues regarding the implementation of ADS and other information systems initiatives.
- Oversee procurement ordering process. Make sure all necessary supplies are ordered in a timely fashion.
- Provide information to Contract Management to ensure accuracy of their files.
- Participate in genetics research and cancer database development working groups.
- Enhance communication with Hematology Oncology Department, and develop final system for physician rotations in the BCC.

**SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING  
PERIOD**

*Patient Service Representatives  
Grimes, Jenkins, Lozoya, Williams*

- Continued organization of front-desk procedures
- Assisted in development of standard operating procedures.
- Processed and interviewed patients through CHCS and designated forms, obtained and updated all patient demographic information and ensured completion of forms.
- Obtained and verified pertinent insurance information utilizing available forms.
- Obtained third party insurance forms from physicians at end of each visit.



- Required identification card from each patient and imprinted all clinic forms pertinent to that patient.
- Received patients and incoming telephone calls/inquiries, determined priorities and referred to the proper source.
- Explained clinic procedures to patients.
- Retrieved/returned Mammogram films daily.
- Ensured completion of incomplete patient records and third party insurance forms.
- Set up records and maintain filing system for paperwork associated with each patient record.
- Ensured that all documents processed are in accordance with department standards.
- Filed all forms in designated order in patient record.
- Labeled files for permanent shadow files.
- Scheduled and coordinated front desk procedures in accordance with department policy.
- Identified process problems and helped develop suitable solutions.
- Oriented new support team members and clinical team staff to office routine.
- Participated in team planning to assure team members meet team quality standards.
- Maintain department standards of productivity.
- Notified physicians the day before they are scheduled for clinic; let them know approximately how many patients they will have.
- Attended classes for the Ambulatory Data System (ADS), a new computer system for collecting outpatient workload data

*Balintona*

- Developed social work information packets to be provided to newly diagnosed patients. The information provided to patients discusses emotional impact of diagnosis, concerns of spouses/loved ones, body image, intimacy issues following surgery (mastectomy or lumpectomy), and adjustment to life after cancer diagnosis.
- Obtained approval from the Clinical Investigation Department at the National Naval Medical Center to run the research
- Completed a pilot study composed of spouses of breast cancer
- Started a new support groups in the Breast Care Center which is a Stage I and Stage II Breast Cancer Survivors Group. This new group is a closed group that meets for five consecutive sessions and addresses emotional issues that arise during breast cancer treatment.
- Addressed the psychosocial status, mental status and patient/family concerns in the Breast Care Center



- Development of the Social Work Assessment that can be completed on computer. This enhances the social workers efficiency because of time savings and rapid chart documentation.
- Worked closely with the BCC Nurse Case Managers to provide seamless care to patients. This includes daily integration and discussion of services provided to ensure patient care continuity and enhanced patient satisfaction.
- Liaison with the National Naval Medical Center Social Work Department. This included integration with the Social Work department with the signing of a Memo of Understanding signed by the director of Breast Care Center and NNMC Social Work Department during the current reporting period. This ensures that social work coverage will be provided in absence of BCC social worker (during a time of unplanned illness or planned vacation days).

*Blankenship*

- Provide comprehensive care to patients with diseases of the breast.
- Provide feedback on patient care processes and suggest improvements.

*Durand*

- Have assisted Digital Equipment Corporation in the development of the Task Manager.
- Have worked collaboratively with staff members on other units, and have successfully established a system of making appointments in advance of receipt of consults.
- Have successfully entered an additional 28 positive patients , and an additional 40 biopsy patients to the Care Manager.
- Have resolved critical issues between patient and providers concerning their methods of treatment.
- Have been influential in the tracking of patients with positive mammography's that would be otherwise lost within the system.
- Initiated 4 Standard Operating Procedures for the referrals of patients to: Physical Therapy, Radiation Oncology, Hematology oncology and Plastic Surgery.
- Attended Evelyn Woods Program for Reading Dynamics.
- Have successfully oriented the new Nurse Case Manager to her position.
- Prepared and presented the role of the Nurse Case Manager to the Interns and Residents and others in the Surgical dept.
- Demonstrated on an ongoing basis the functions of the "Care Manager" (developed in collaboration with Digital), to various visitors and dignitaries.



- Was influential in the pre-op preparation of the surgical procedure for a Naval Admiral. Accomplished the Certification of the Plastic Surgeon, the ordering of 6 prostheses, and setting up of the surgery schedule all one day before the procedure.

*Richman (Fields)*

- Performed a variety of mammograms, stereotactic biopsies, needle localizations and ultrasound procedures.
- Interfaced with mammography doctors to increase knowledge in the areas of mammography and breast disease.
- Became more familiar with the BCC personnel.
- Obtained CPR certification.
- Received 3 CEU from Eastman Kodak for film demonstration.
- Established QA protocol for the stereotactic biopsy machine.

*Higgins*

- Refined breast self exam teaching and monitored patient's teaching needs
- Enhanced nursing knowledge base on breast cancer issues
- Further developed personal computer skills
- Attended seminars/conferences on breast cancer issues and professional nursing issues
- Functioned as a team member to develop nursing and documentation
- Improved patient flow processes in the clinical area
- Became involved with the nursing research aspect of the BCC
- Continued to orient new staff members to patient flow processes and forms
- Coordinated patient flow activities in the clinical areas with patients, nurses and doctors
- Stocked all exam rooms and clinical areas, appropriately
- Coordinated all FNAs and procedures and notified the Nurse Case Manager of such procedures
- Triaged telephone calls and walk-ins. Also organized triage area and files
- Processed linen and hazardous material
- Developed results folder and cancer follow-up patient package
- Participation and case study presentation at BCC conference
- Random Chart Review of 60 patients with family history of breast cancer for preventative to Genetic Research meeting
- Telephone Triage Call Log data collection and presentation to nursing staff
- Met with the Technical Assistant to discuss data base for research
- Met with Digital to discuss potential technical needs and resources of the Research Nurse



- Discussion with Nurse Manager and Head of General Surgery regarding expectations of the Research Nurse

*Lopez*

- Continued responsibility as the designated safety representative of the BCC.
- Maintained credentialing data base on all Geo-Center employees.
- Planned and instituted staff education calendar and events.
- Functioned as Clinical Educator providing teaching on breast self examination, pre- and post-operative instruction and breast cancer.
- Functioned as relief ambulatory care nurse providing breast self exam teaching, assisting the physicians with physical exams, procedures, and scheduling of diagnostic test when needed.
- Started work on the development of SOP manual.
- Implemented patient home care instruction brochure and introduced brochure to residents, interns, 5-East and APU.
- Developed and implemented Education Resource Room brochure which contains log of videos available to patients.

*McIntyre*

- The above technical objectives were met during the current reporting period.
- Coordinated mammography scheduling.
- Supervised other mammography personnel.
- Obtained mammography statistical data for FDA purposes.
- Obtained credentialing information on radiologists.
- Tracked 6 month follow-up patients with outcome analysis via BCC Task Management Tool.
- Correlated mammography and pathology findings via Composite Health Care System (CHCS).
- Became more proficient utilizing the Digital computer system.
- Assisted with passage of 1996 FDA Mammography inspection

*Portee*

- Coordinated patient flow activities.
- Performed professional nursing assessments.
- Provided Breast Self Examination teaching.





- Prepared patient charts appropriately with medical, lab, and x-ray reports.
- Assisted physicians with procedures.
- Provided physical and emotional support to patients.
- Collaborated with social service, nurse case managers, clinical nurse educator, physical therapist and physicians to ensure exceptional patient care.
- Disposed of linens and hazardous wastes appropriately.
- Prepared needle and syringe inventory document
- Read literature on breast cancer and its treatment.

*Prindle*

- Coordinated patient flow activities
- Acted as relief nurse manager for the BCC on several occasions
- Collaborated with many physicians concerning unscheduled patient visits
- Performed professional nursing assessments
- Provided BSE and biopsy teaching
- Triageed patient phone calls and made telephone consults to physicians
- Prepared patient charts appropriately with medical, lab, and x-ray reports
- Entered physician orders into the computer
- Assisted physicians with many procedures done in the BCC
- Provided physical and emotional support to patients
- Collaborated with social service, nurse case manager, clinical nurse educator, physical therapist and many physicians to ensure exceptional patient care
- Developed new role as Team Leader
- Managed/Supervised new nursing staff for the ambulatory care area
- Developed BCC medical reference library list
- Completed algorithm format for clinical process

*Snee*

- Provided case management for a minimum of 45 patients and their families
- Trained in the use the "Care Manager" software
- Suggested and implemented useful changes in the care manager software
- Helped to educate patients and families on breast cancer
- Provided emotional support to women from diagnosis, to completion of breast cancer treatment
- Collaborated with multiple disciplines to arrange for patient care
- Developed useful methods for managing many varied and complex patients



- Taught many new cancer patients about breast prosthetics and assisted them in obtaining the prosthetic
- Attended tumor board meetings and was prepared to give additional information concerning breast cancer patients if required or requested by physicians
- Attended inservices to educate nurses in chemotherapy protocols
- Collaborated with staff on the development of a cancer database

*Taylor*

- Worked with the Patient Service Representatives to learn the front desk process and procedures
- Updated the Standard Operating Procedures for the Patient Service Representatives
- Assisted Patient Service Representative as well as nurses when/where needed.
- Assumed responsibility for the procurement process and orders.
- Provided end user support for computer system.
- Assisted with the setup and desktop publishing of several conferences.
- Performed data entry of research questions.
- Performed word processing for the center's staff.
- Organized supplies to allocate more usable space.
- Ensured a smooth correspondence flow/distribution.
- Provided structure and flow for Cancer Database.
- Typed military performance evaluations and standard forms.
- Maintained the electronic filing system to organize the out going correspondence flow.
- Wrote patient correspondence for appointment and medical issues.
- Generated supply reports.
- Worked with Digital personnel to install, upgrade, and configure BCC computer systems.

*Vaughn*

- Provided assistance to staff requesting help with mammography films.
- Organized log book to improve film tracking.
- Devised a new way to disseminate films to patients via TICS computer input.
- Designed release form for patients signing out mammogram films.



*Wallace*

- Developed mechanism for briefing new residents/interns on BCC operations.
- Coordinated administrative activities of the BCC. Acted as administrator during the absence of the Administrator and Nurse Manager.
- Worked with Nurses to develop filing guidelines for patient charts.
- Supervised research of alternative method for patient charting.
- Worked on Strategic Plan.
- Assessed Information System needs, including Hardware and Software, for immediate and future needs.
- Managed schedule templates for BCC attending physicians in five subclinics.
- Completed initial process of ensuring accuracy of Contract Management Department files, developed long-term plan for keeping files up-to-date.
- Developed system for budget management expenditure tracking. Researched Patient level accounting.
- Successfully mentored administrative intern for six weeks.
- Expanded use of CHCS standard reports.
- Revised budget based on new funding, coordinating with Budget Department to match records.



## GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

### *Patient Service Representatives Grimes, Jenkins, Lozoya, Williams*

- Become proficient in the use of ADS.
- In effort to continuously improve quality, streamline and organize front-desk procedures.
- Coordinate scheduling with other clinics for smoother follow-up visit for the patient.

### *Balintona*

- Pass the LCSW-C exam, which will enhance the social worker's professional practice and expertise within the field of social work.
- Continue working relationship with the American Cancer Society to bring programs that address psychosocial issues related to cancer to the Breast Care Center.
- Enhance Care Manager (computer program utilized in BCC for patient care) usage for patient care integration. This objective will include daily documentation on the Care Manager and continued discussion with computer programmers to make it compatible to social work service provision.
- Liaison with Dr. Ken Miller, Nursing Researcher, for further feedback and input on the research proposal.
- Provide social work services to patients by addressing psychosocial status, mental status, patient and family concerns

### *Blankenship*

- Continue to provide care for patients with both benign and malignant diseases of the breast at the Breast Care Center. Increase the number of patients seen in the Breast Care Center to approximately 500 patient visits each month.
- Improve patient charting process.
- Data collection system and other patient care processes.



*Durand*

- Continue to work in collaboration with Digital to improve the "Care Path Manager" and assessment of the database.
- Continue to develop the Standard Operating Procedures for the position of the Nurse Case Manager.
- Provide Pre-op teaching in conjunction with the physician, for the patients and their families regarding alternative treatments for breast cancer. (Required by the dept. of Health and Mental Hygiene.)
- Distribute educational material for treatment options to patients and their families.
- Initiate, with the help of Digital, a method to measure outcomes via the Care Manager. Also to develop variances to use in conjunction with these outcomes.
- To participate in regular peer review/staff meetings regarding how the unit functions, to improve patient care.
- Continue to educate other staff members about Case Management and the unique needs of the Case Managed patient populations.

*Richman (Fields)*

- Obtain mammography certification.
- Attend an educational mammography seminar.
- Broaden my knowledge of breast diseases and mammography.

*Higgins*

- Continue to enhance nursing knowledge base on breast cancer issues
- Continue to further develop personal computer skills
- Continue to attend seminars/conferences on breast cancer issues and professional nursing issues
- To define and develop the roles and responsibilities of the Research Nurse position
- To complete the orientation process of the Research Nurse position
- To initiate nursing and clinical protocol development in the BCC
- Utilization of the Care Manager to monitor trends in the BCC
- Working with the multidisciplinary team in writing the protocol for BRCA1 and BRCA2 testing



*Lopez*

- Continue responsibility as safety representative
- Continue to function as Clinical Nurse Educator providing teaching to patients and their support persons.
- Continue to function as relief Ambulatory Care Nurse.
- Participate in wellness programs.
- Identify needed materials and supplies for procurement.
- Continue to work on SOP manual as work schedule allows.

*McIntyre*

- Continue to perform nursing and managerial duties, as described above.
- Continue to obtain mammography statistical data for FDA purposes on a monthly basis.
- Track 6 month follow-up patients with outcome analysis via BCC TaskManagement Tool.
- Track stereotactic core biopsies per radiologist for new ACR credentialing purposes.
- Attend nursing/management conferences when available.

*Portee*

- Continue to enhance my education in breast cancer and its treatment.
- Continue to improve patient flow management.
- Continue to improve computer skills.
- Attend a seminar/conference related to breast cancer.
- Continue to participate in multi disciplinary meetings.
- Continue to learn the teaching of biopsy and APU.

*Prindle*

- Will develop breast care center medical reference library
- Will continue to enhance education in breast cancer and its treatment
- Will continue development of nursing protocols and quality assurance documents, especially in the area of telephone triage
- Will continue to develop Team Leader Role and patient flow activities
- Will continue to improve computer skills
- Will attend a seminar/conference related to breast cancer on 9/6/96



- Will continue to participate in multidisciplinary meetings
- Will improve upon and fine tune presentation on ambulatory care nursing for future presentations
- Will improve expertise in patient education for APU patients having breast surgery
- Will attend a seminar for Health Professionals in utilizing the Internet
- Will continue to develop new processes to better enhance clinic management

*Snee*

- Will continue to improve skills as a nurse case manager
- Will assist in the development of a breast cancer database
- Will develop concise methods to manage multiple patients
- Will continue to enhance education in breast cancer and it's treatment
- Will continue to improve computer skills
- Will attend a seminar/conference related to breast cancer
- Will continue to participate in multidisciplinary meeting
- Will establish guidelines for case management follow up after the acute stage of diagnosis and treatment of the breast cancer patient

*Taylor*

- Continue with streamlining office procedures and processes to improve efficiency.
- Maintain, improve, and/or introduce databases to improve productivity and organization of data.
- Define a personal development program to assure continuing professional growth.

*Vaughn*

- Continue with duties as described above.
- Reduce turn around time for mammogram films returned to the department from the BCC, General Surgery Clinic and patients.
- Purge duplicate mammogram folders



*Wallace*

- Continue to nurture relationship with the Radiation Oncology Clinic and the Medical Oncology Clinic.
- Continue participation on genetics research and cancer database working groups.
- Research possibility of ad hoc reporting in CHCS.
- Continue to coordinate administrative activities of the BCC.
- Complete research on patient level accounting system, begin implementation if appropriate.
- Complete research on alternative methods for patient charting to reduce amount of paper and select a system.
- Complete Strategic Plan, Information Systems Plan, and Marketing Plan.
- Develop process for coordinated budget/accounting system between BCC and Budget Department.





### **III. NDRI, Great Lakes, IL and NDRI Detachment, Bethesda, MD**

#### **A. DENTAL DISEASES-RELATED RESEARCH**

##### **DESCRIPTION OF WORK TO BE PERFORMED**

###### *Beck*

- Provide technical assistant with ongoing Immunology research projects. Participate in linkage analysis project with Molecular and Epidemiology of NIH. Maintain and upgrade the laboratory such that the research experiments are carried out smoothly. Maintain and record proper technical procedures and data produced for each experiment

###### *Jones*

- Senior Research Scientist. Responsible for the Molecular Biological and Molecular Genetic aspects of the projects. This includes the development, evaluation and refinement of molecular biological research protocols.

###### *Turner*

- To work as a Senior Scientist in the Basic Sciences Department of the Naval Dental Research Institute, and as the Principal Investigator on 61152N project, "Antimicrobial activities of polymorphonuclear granule components in human periodontal diseases". This project has been approved for funding from 1 October 1993 to 30 September 1996. The Principal Investigator heads a research team consisting of Mr. Ernest Pederson, HM2 Carolyn Merritt and HM3 Colin Glynn.

###### *Lamberts*

- To assist as an editorial consultant in the preparation or review of manuscripts to be submitted for publication.
- To aid in the preparation of research presentations (such as posters) for scientific meetings, in the review of research proposals, research communications (letters, rebuttals), etc.



*Miller*

- Senior Research Scientist and Group Supervisor. Responsible for all aspects of Immunological, Microbiological, and Tumor Biomarker activities within the Naval Dental School. This includes the development and supervision of research protocols, dental resident mentoring activities, instruction of courses in dental microbiology and dental immunology, serving as a link between NIH sponsored research and Naval Dental Research programs, and troubleshooting of research programs, computers, instrumentation and equipment.

*Ovsey*

- Scientist III conducting research support to the mission of the Naval Dental Research Institute. Conduct immunological and microbiological research in the areas of oral diseases and periodontal distress.

**TECHNICAL OBJECTIVE FOR THIS REPORTING PERIOD**

*Beck*

- Continue gathering samples of stimulated lymphocytes for RNA analysis study.
- Begin experiments on gathered samples by isolating RNA's and subsequently converting to cDNA's and evaluating the PCR products via gel sequencer.
- Grow and maintain various fibroblast cell lines.
- Assist Molecular and Epidemiology group of NIH with the Cleft-Lip Palate (CLP) disorder linkage analysis study.

*Jones*

- The Naval Dental Research Institute, Detachment Bethesda, the National Naval Dental Center and the National Institute of Dental Research, National Institutes of Health entered into an agreement to identify, refine, develop, test and evaluate molecular, genetic, and cellular biomarkers for the epidemiological study of premalignant and malignant oral lesions and to characterize these biomarkers for their potential use in the identification of individuals at risk. Samples of normal tissue and tissue derived from oral lesions for evaluation are to be provided by a study entitled "Biomarkers for Oral Cancer," which involves recruitment of subjects for biopsy of



oral lesions and collection of epidemiological data at collaborating Veterans Administrations hospitals. Geo-Centers, Inc. is responsible for providing necessary personnel for this project to work principally in the areas of evaluation of predisposing risk factors such as genetic polymorphisms in genes encoding xenobiotic metabolizing enzymes, natural variation in human leukocyte antigen (HLA) and infection with human papillomavirus (HPV). Premalignant and malignant tissues are to be evaluated for loss of function of the anti-oncogene p53 and for changes in the expression of the *ras* oncogene. I have been recruited to provide the necessary expertise in molecular biology and molecular genetics for the evaluation and analyses of these biomarkers. I expect to devote considerable effort to this program which is expected to have a duration of a minimum of 4 years.

*Turner*

- To repeat and verify procedures for testing granule components against *Treponema denticola*. The effectiveness of Cathepsin G against this oral pathogen was verified along with the lesser microbicidal activity of lysozyme.
- An abstract of this work has been prepared and sent to the International Association for Dental Research for inclusion in the annual meeting of that organization in Orlando, Florida, in March 1997. In addition, a manuscript is now in preparation for submission to a scientific journal (Infection and Immunity). Work on this manuscript should be completed in October, 1996 and submitted at that time.

*Lamberts*

- To complete work on the preparation of the manuscript by E. Pederson et al. on "Heavy Metal Pollutant Removal from Dental Operatory Waste Water".
- To continue work on a manuscript of D. Turner et al. on "Microbicidal effects of neutrophil-granule components on *Treponema denticola*".
- To assist Naval Dental Research Institute (NDRI) investigators editorially whenever requested.

*Miller*

- Relative to the 2-D PAGE evaluation of components of gingival crevicular fluid, to await IR funding of this program.
- Relative to the program entitled "Evaluation of disproportionate expression of T-cell receptor V $\beta$  regions in lymphocytes from patients with advanced periodontitis",



mRNA's from cultured peripheral blood mononuclear cell preparations will be transcribed to cDNA and the cDNA's subjected to analysis for TCR V $\beta$  chain expression by PCR and quantitation on an ABI 273 fluorescence sequencer.

- Relative to the project "Cytokine production by polymorphonuclear leukocytes resident in periradicular and periodontal lesions", a summary of results will be presented at the Annual Meeting of the American Association of Immunologists in New Orleans in June, 1996 and a final manuscript completed and submitted for publication.
- Relative to the program entitled "Biomarkers for Oral Cancer," work will proceed in developing appropriate methods to identify polymorphisms in genes (such as CYP1A1) involved in the metabolic activation or detoxification of tobacco. This work will be supervised by Dr. John Jones. In addition, work in the area of p53 evaluation and papilloma virus DNA identification in oral lesions will continue.
- Work will begin on a project designed to evaluate antibacterial activity of currently used endodontal medicaments. It is anticipated that six compounds will be evaluated against at least four anaerobic organisms generally found to be associate with endodontal infections.

*Ovsey*

- Identify, order and sustain the required equipment necessary to perform mercury analysis by Perkin-Elmer method (PE) 245.1A using PE5100 PC Atomic Absorption Spectrophotometer, FIAS400 - flow injection system, and AS-90 - auto-sampler.
- Optimize the instrument, attain the Initial Demonstration of Competence (IDC), perform the Method Detection Limit (MDL) estimate (40 CFR App. B part 136), and approximate the Linear Dynamic Range to define the upper limit of mercury analysis. Analyze Quality Control Samples (QCS) quarterly. Enroll and participate in all available Water Pollution (WP) and Water Supply (WS) studies. Passing the WS study is required to be eligible for the IEPA certification.
- Develop a draft of the laboratory Quality Assurance Plan (QAP). The final QAP will be submitted to the IEPA with the Laboratory Questionnaire and WS results.
- Prepare the laboratory toward the eventual IEPA laboratory inspection.
- Aid NDRI in the facilitation of the identification of this organization's role in a program to aid the naval dental facilities with the improvement of quality of dental waste water



## SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD

### *Beck*

- Lymphocytes subjected to the antigens such as Td 33521, Td 33520, and anti CD3 were gathered for RNA isolation and PCR studies. Cells in each group were frozen in liquid nitrogen for future experiments of RNA analysis.
- RNA's were isolated from frozen lymphocytes and reverse transcribed to make cDNA's. Then, sequences of cDNA's (specific for loci of interest) were amplified and tagged with fluoroscenated markers using polymerase chain reaction (PCR) technique. These PCR products were visualize by running PCR products through acrylemide gels on gel sequencers.
- Several cell lines are being maintained for continuous growth. These cells, gingival and OT-1 (tumor cell line), were subjected to various growth factors and bacterial cell components to observe modifications at nuclear level. RNA's were isolated from these cells after two days of incubations and stored for future reverse transcription and PCR experiments. Continued to participate in NIH Molecular and Epidemiology experiments. This study deals with the inherited genetic disorder, CLP. Primarily, linkage analysis is done by amplifying DNA loci that may be associated with this inheritable disorder via PCR and running gels to visualize the amplified gene sequences.

### *Jones*

- Relative to the program entitled "Biomarkers for Oral Cancer," I have identified a panel of genes for the analysis of inter-individual genetic variation. Material available for analysis will be limited and must be shared among several investigators. I have, therefore, invested considerable effort in the optimization of methods for the analysis of these genes using the polymerase chain reaction (PCR) technique. A large collection of oligonucleotides and restriction enzymes has been established for these analyses.
- Optimization of techniques to identify single basepair alterations in tumor-derived DNA have also been completed. Single-strand conformation polymorphism (SSCP) analyses have been carried out on DNA obtained from a small sample of oral tumors with very promising results.
- Designed a novel human papillomavirus molecular standard for use as an internal control for the determination of HPV infection via PCR. This molecular standard will



facilitate the determination of PCR reaction status, sensitivity of the HPV assay and quantitation of template (target) DNA concentration and viral copy number. The standard has been designed to be used in the "accepted" Manos protocol for the detection of HPVs 06, 11, 16, 18 and 33 which are most closely associated with cancer. The standard has been specifically designed for use with fluorescent-label primers and automated detection equipment.

Publications/Presentations, etc.

- Predisposing Genetic Factors for Oral Carcinogenesis - A Review. (tent.) J. E. Jones. Manuscript in Preparation

*Lamberts*

- Worked on revising the format of the manuscript of E. Pederson on "Reducing medium for the cultivation of anaerobic *Porphyromonas gingivalis* under aerobic conditions". This paper had been submitted to a journal for publication but was not accepted, so will be resubmitted to another journal.
- Continued work on revisions of the manuscript of S. Schade et al. on "Mitogenic Activity in the Outer Membrane of *Treponema denticola*", and on preparation of the manuscript of E. Pederson et al. on "Heavy Metal Pollutant Removal from Dental Operatory Waste Water".
- Reviewed the galley proofs of a manuscript of J. Simicek that is ready for publication.

*Miller*

- Relative to the development of a 2-D electrophoresis procedure to study components of gingival crevicular fluid from individuals with severe periodontitis, preliminary studies have been incorporated into a research proposal that has been submitted for IR monies. This proposal has been submitted with Dr. M.M. D'Alesandro, MSC, USN as principle investigator in order to follow current guidelines. Although funding was not forthcoming during the first round review, we await a final decision from the second round review.
- Relative to the program entitled "Evaluation of disproportionate expression of T-cell receptor V $\beta$  regions in lymphocytes from patients with advanced periodontitis" (Work Unit: 0601152N.MR00001.001-0063), all control total RNA isolations as well as cDNA synthesis for all normal samples have been completed. In addition, cDNA's have been titrated to equalize C $\alpha$  pcr products using the ABI 272 sequencer. Total RNA is also now available from 7 individuals with advanced periodontitis.



- Relative to the project "Cytokine production by polymorphonuclear leukocytes resident in periradicular and periodontal lesions" (Work Unit: 0601152N.MR00001.001-0063); a paper has been presented at the Annual Meeting of the American Association of Immunologists in New Orleans this past June, 1996. A final manuscript is nearing completion and should be submitted during the next quarter.
- Relative to the program entitled "Biomarkers for Oral Cancer," work has been progressing toward the development of a dot blot procedure for identification of human papillomavirus DNA in oral cancer biopsies.
- Work Unit: 0601152N.MR00001.001-0063. Long term frozen storage of lymphocytes. One manuscript titled "Studies of Proliferative Responses by Long-Term-Cryopreserved Peripheral Blood Mononuclear Cells to Bacterial Components Associated with Periodontitis" has been accepted for publication in Clinical and Diagnostic Laboratory Immunology.
- Relative to the project Evaluation of the Influence of Superantigens and Polyclonal B-cell Activators in Periodontal Disease (Work Unit 0601152N.MR00001.001-0063) one manuscript reporting these results titled "Studies of immunomodulatory and superantigen activities of bacteria associated with adult periodontitis has been accepted for publication in Periodontology."
- Relative to studies evaluating antimicrobial activity of dental materials which was completed about one year ago a manuscript titled "Antimicrobial activity of dentin bonding systems and glass ionomers" has now been accepted by the Journal of Operative Dentistry.
- Relative to studies designed to evaluate antibacterial activity of currently used endodontal medicaments, Vitapex® and Calasept® and a variety of controls were evaluated against *S. mutans*, *S. sanguis*, *S. aureus*, and *E. coli*.
- A project has recently been initiated to study cytokine production by oral fibroblasts. This study extends a previous study designed to look at the influence of various growth factors on wound healing. Various gingival and pulpal fibroblasts have been obtained and cultured with bacterial stimulators. Total RNA has been isolated and used to prepare cDNAs by reverse transcription. In addition, primers have been identified for use in a PCR based semiquantitative analysis of mRNAs for IL-1 and IL-6.

#### Publications

- G. A. Miller, T. DeMayo and J.Hutter. 1996. Production of interleukin-1 by polymorphonuclear leukocytes present in inflammatory periradicular tissues., *Journal of Endodontics*. 22:346-351.



Presentations

- Miller, G.A., M.M. D'Alesandro and G. Euler. 1996. Production of interleukin-6 by polymorphonuclear leukocytes in gingival and periradicular tissues. Annual Meeting of the American Association for Immunology, New Orleans, La.

*Ovsey*

- Ordered and received all equipment necessary for the performance of mercury analysis by the Perkin-Elmer method (PE) 245.1A. Satisfied the additional equipment requirements of the IEPA Certification Program. Continued to purchase laboratory supplies and services based on maintenance and experimental needs.
- Completed the method validated in accordance with IEPA and EPA requirements.
- Maintained this laboratory's capability to analyze mercury in water and waste water samples.
- The MDL for the EDL System 2 lamp was estimated at approximately 0.07 micrograms per liter. This means that, if a drop of mercury weighing 1 gram was dissolved in a pool of water with the volume of 14.3 million liters of water, this laboratory would be able to detect such mercury concentrations. The Illinois mercury discharge requirements are 7.1 times higher than this laboratory's MDL (i.e., 0.5 micrograms per liter). Estimated the MDL's for Silver and Copper analyses using the Transverse Heated Graphite Analyzer. These MDL's were 0.2 and 0.5 micrograms per liter for Silver and Copper respectively.
- The LDR estimate was performed to confirm the upper bound of linearity of this analysis method.
- The QCS analyses were performed to periodically confirm this laboratory's analyses with an externally prepared and NIST Certified mercury standard.
- Participated in the WS037 samples. The results of this study are expected to be released by the U.S. EPA in the September, 1996 time frame (U.S. EPA Region V office released preliminary results that indicate that our results [8.08 micrograms Hg per liter] agreed with their median [7.64 micrograms per liter - unofficial value based on the results of 15 laboratories in Region V]).
- Trained NDRI's instrument operator to perform mercury analysis. The operators competence will be verified by the analysis of the WP036 Mercury sample. The result for this analysis by the NDRI's instrument operator (in training) will be available in the November 1996 time frame.





- Developed the NDRI's Environmental Analytical laboratory Quality Assurance Plan (QAP), pending approval by the IEPA towards laboratory inorganic mercury certification.
- Continuing support efforts of the NDRI's research objectives, by training Research Analysts, providing technical input, processing and analyzing samples, and developing and implementing new metals analysis techniques, will aid in the achievement of NDRI's research mission. This research continues to produce on an on going basis very important and relevant data that is essential for research publications and workable long term solutions.
- Co-author of a paper which will be submitted to JADA in the next quarter: Pederson, E.D., Stone, M.E., Ovsey, V.G., and Batchu, H.R.: Mercury removal from dental operatory waste water by polymer treatment (paper not included).

#### **GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD**

##### *Beck*

- Main objective of this upcoming quarter is to continue with NIH-CLP project.
- Continue gathering necessary samples for the RNA analysis.
- Continue isolating RNA's and convert to cDNA and identify interested locus via PCR.

##### *Jones*

- Relative to the program entitled "Biomarkers for Oral Cancer," work will proceed on the development of appropriate methods for the identification of polymorphisms in genes resulting in increased cancer risk. We anticipate the arrival of DNAs and/or tissue samples from the oral cancer study and will begin processing them immediately.
- We are working toward the development of a comprehensive database for the maintenance of data generated in this study. We will be working closely with individuals from the NIH expert in database design and maintenance.
- Will become involved in the investigation of bacterial stimulator-mediated induction of expression of IL-1 and IL-6 specific mRNAs. Will explore the development of an internal standard molecule to facilitate the quantitation of specific cytokine gene expression.



*Turner*

- This project is scheduled for termination on 30 September 1996.

*Lamberts*

- Complete, if possible, work on the two manuscripts of E. Pederson et al., the manuscript of S. Schade et al., and the manuscript of D. Turner et al.
- Assist NDRI investigators editorially whenever requested

*Miller*

- Relative to the 2-D PAGE evaluation of components of gingival crevicular fluid, to await IR funding of this program. Should funding not be forthcoming the project will be halted.
- Relative to the program entitled "Evaluation of disproportionate expression of T-cell receptor V $\beta$  regions in lymphocytes from patients with advanced periodontitis", mRNA's from remaining cultured peripheral blood mononuclear cell preparations will be transcribed to cDNA and the cDNA's subjected to analysis for TCR V $\beta$  chain expression by PCR. It is expected that the majority of this project will be completed in the next quarter.
- Relative to the project "Cytokine production by polymorphonuclear leukocytes resident in periradicular and periodontal lesions a final manuscript is nearing completion and will be submitted for publication.
- Relative to the program entitled "Biomarkers for Oral Cancer," work will continue relative to the identification of human papillomavirus DNA and efforts will begin to establish a PCR/OLA procedure for identification of specific alleles in various human DNA samples.
- Work will continue on a project designed to evaluate antibacterial activity of currently used endodontal medicaments. It is anticipated that Vitapex® and Calasept® will be evaluated against anaerobic organisms such as *P. intermedia*, *P. gingivalis*, and *A. viscosus* during the next quarter.
- Work will continue toward the quantitation of IL-1 and IL-6 specific mRNA in oral fibroblasts cultured in the presence of various oral bacterial stimulators. In addition, a collaborative effort between our laboratory and that of Dr. M.M.Sholley, Department of Anatomy, Medical College of Virginia will be initiated to look at the influence of bacterial stimulators on cytokine production by endothelial cells in culture.
- Begin teaching Oral Microbiology for Naval Dental Residents.



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*Ovsey*

- N/A



GEO-CENTERS, INC.

#### IV. NMRI TOX/DET Dayton, OH

##### A. TOXICOLOGICAL STUDIES

#### DESCRIPTION OF WORK TO BE PERFORMED

##### *Abbas*

- Member of the Pharmacokinetic group.
- Project Director for the largest project (Trichloroethylene Biologically Based Health Risk Modeling) within Tri-Service Toxicology Consortium. The project is funded by Strategic Environmental Research and Development Program (SERDP) \$900K per year.
- Responsible for meeting customer needs, interact with high-level federal government scientists, brief and provide monthly and quarterly status reports to the Science Advisory Board and Executive Chair of the Strategic Environmental Research and Development Program (SERDP).
- Collaborate with Scientists from United States Environmental Protection Agency (USEPA), Wayne State University, Creighton University, University of Wurzburg (Germany), and Zeneca (United Kingdom) to investigate Human toxicity of TCE.
- Lead multiple research teams to development methods, explore pharmacokinetics, pharmacodynamics, metabolism, metabolic pathways of trichloroethylene (TCE) and its metabolites, chloral hydrate (CH), trichloroethanol (TCOH), trichloroethanol glucuronide (TCOG), trichloroacetic acid (TCA) and dichloroacetic acid (DCA).
- Develop a comprehensive Biologically Based pharmacokinetic model for TCE and five metabolites.

##### *Ademujohn*

- The purpose of the neurobehavioral laboratory coordinator at NMRI/TD is to provide technical support to various aspects of ongoing on-site projects in neurobehavioral research. During this quarter the coordinator has been and will be involved in neurobehavioral testing for the effects of simulated stress factors relating to the Gulf War Syndrome on animal models via computer-aided qualitative and quantitative methods. The coordinator also supervises animal training protocols for upcoming pharmaceutical exposure studies.



*Briggs*

- General manager and Senior Contractor representative for Geo-Centers, Inc. for the NMRI contract at the Toxicology Detachment. He also serves as a toxicologist and performs research as an Associate Investigator. He is responsible for collaborating the Geo-Centers, Inc. resources in support of the toxicology research projects in support of the Toxicology Detachment mission. Dr. Briggs responds to taskings from the Officer In Charge which includes assuring compliance with the Quality Management Plan.

*Carraci*

- Group Supervisor I/Scientist II.
- Member of the Pharmacokinetics Group.
- Study Director for project, Physiologically Based Pharmacokinetic Modeling of Cardiac Sensitizing Chemicals in Rats.
- Perform laboratory experiments to support the Halon 1301 Replacement Toxicity project.
- Perform inhalation experiments for the Total Petroleum Hydrocarbon (TPH) Project.

*Confer*

- Scientist I.
- Member of Hazard Assessment Group.
- Member of Good Laboratory Practices Process Action Team (GLP-PAT).

*Connolly*

- Cataloging print and non-print materials for circulation
- Ordering and maintaining serials collection, including claiming missing issues
- Handling reference questions
- Providing interlibrary loan assistance
- Locating needed materials in other libraries
- Preparing book orders



*Garrett*

- Scientist II.
- Member of Pharmacokinetics Group.
- Member of Dermal Penetration Project Team.
- Member Trichloroethylene Project Team.
- Room Monitor.

*Geiss*

- Group Supervisor II/Scientist III.
- Group Administrator for the Pharmacodynamics (PD) Technical Area Group, Tri-Service Toxicology Consortium.
- Methods and protocols development for the molecular biology laboratory.
- Ensure PD support is provided to project leaders.
- Perform ongoing molecular biology analysis for project experiments.
- Participate in project planning.

*Grabau*

- Senior Scientist I.
- Member of Pathology Technical Area Group.
- Provide scientific image processing and analysis to multiple ongoing research efforts.
- Member of TriService Marketing/Program Development Team.

*Horton, Rix*

- Maintain Computer Network
- Maintain and upgrade individual Desktop and Laboratory Computers
- Provide answers, support and expertise in correcting computer problems, including all peripherals attached to these systems
- Develop comprehensive program for maintaining system integrated and reliability through back-up procedures, documentation, and redundant systems
- Continue conversion of Windows for Workgroups (WFW) systems to Windows 95
- Continue to update information Databases HMIS, IRIS, Medline and Toxline
- Maintain and Update NMRI Web page and internet services
- Organize Media, Manuals and Spare Parts



- Prepare Automated System Decision Papers for procurement of new computer systems, software and peripherals

*Jung, Narayanan, T.K.*

- During the past quarter, the work carried out by this group was:

Trimethylolpropane (TMPP) Evaluation

- Measurement of the amounts of amino acids and neurotransmitters present in brain homogenates using an HPLC method
- Begin culturing the mouse neuroblastoma cell line N2-A

Cell Model Project

- Collection of the metabolites of acetamidophenol from the urine of rats that were dosed with 0.2 g/kg.
- Preparation of four pre-proposals for future work and the abstract to be sent to SOT this Spring

*Kimmel, Reboulet*

- Assist in inhalation project support including exposures and toxicology expertise
- Initiate startup activities for Adult Respiratory Distress Syndrome project, including facility modification
- Use chemistry and computer background to assist principal investigators in conduct of their research projects.
- Assist in building, maintaining, and improving inhalation chambers and support equipment. Provide chemical expertise for the generation, characterization, quantitation, and subsequent Assist in preparation of peer reviewed journal publications, posters, and presentations.

*McDougal*

- Sr. Scientist III/Department Manager.
- Member of the Pharmacokinetic Group.
- Lead basic and applied research in dermal penetration and biologically-based mathematical modeling.
- Responsible for all aspects of quantitative dermal toxicology and dermal risk assessment for Army, Navy and Air Force at Tri-Service Toxicology Center.



- Focal Point for Explosives and Munitions Toxicology.
- Primary investigator on an Air Force Office of Scientific Research project entitled, "Species Differences in Skin Penetration".
- Project Leader for Modular Artillery Charge System (MACS).
- Mathematical modeler for Total Petroleum Hydrocarbon Project.
- Lead multidisciplinary research group (University of Michigan, North Carolina State University, Colorado School of Mines, and University of California at San Francisco) addressing quantitative dermal toxicology for Air Force Chemicals.

*Narayanan*

- Scientist II.
- Member of the Hazard Assessment Group.
- Support combustion and in-vitro toxicology research.
- Support studies involving toxicity evaluation of explosives and propellants.
- Support projects involving toxicity evaluation of vapor phase lubricants.
- Assess neurotoxicity in simulated Persian Gulf War (PGW) exposure in Sprague-Dawley rats.

*Prues*

- Research performed at the Navy Medical Research Institute/ Toxicology Detachment (NMRI/TD) addresses Navy concerns.
- Finding replacements for the current ozone depleting fire extinguishants has prompted the Navy to perform studies on alternative products, such as, the Spectrex fire extinguishant (SFE).
- Because of the toxic effects of chemicals on one's ability to perform their duty, the Navy is involved in trying to ascertain the mode of action of certain toxicants (i.e. TMPP), with which their personnel are likely to come into contact.

*Ritchie*

- Assistant Group Leader for the Neurobehavioral Toxicology Group at the Tri-Service Toxicology Consortium and NMRI/TD and as Associate Principal Investigator (API) for all currently funded neurobehavioral toxicology-related work units (FY97 funding of \$893 K).
- Assist in all areas of program management, budgetary control and procurement, research design, protocol preparation, research supervision, statistical analysis and





preparation of scientific papers and abstracts in the area of neurobehavioral toxicology research. During the current quarter, will have continued research in five areas:

- (A) Neurobehavioral Toxicity Assessment Battery (NTAB): Assessing Animal Responses to Pharmacological Challenge (WU .1605): Predictive validation of the NTAB by comparison of animal and (known) human responses to identical pharmacological challenges on neurobehavioral tests with topographical similarity.
- (B) Improved Methods for Evaluating Performance Deficits Induced by Brief Exposures to High Concentrations of Gases or Vapors (WU .1408): Neurobehavioral effects of exposure to low concentrations of Ozone- Depleting Substances (ODSs) and Non-Ozone Depleting Substance Replacements (ODSRs) of military interest.
- (C) Improved Methods to Evaluate Performance Deficits Induced by Complex Mixtures (WU .1420): Neurobehavioral effects of exposure to low concentrations of single and mixed combustion gases as might be encountered in military scenarios.
- (D) TMPP MECHANISMS OF ACTION: DEVELOPMENT OF NEUROBEHAVIORAL MOLECULARIZATION TECHNIQUES (WU .1516): Development of a number of neuro-molecular (cellular-level analytical techniques for eventual inclusion in the Navy Neuro-Molecular Toxicity Assessment System (The NTAS); anatomical disposition and effects of trimethylolpropane phosphate (TMPP), a potent neurotoxicant produced through the pyrolysis of synthetic lubricants used in military ships and aircraft.
- (E) PERSIAN GULF WAR (PGW) SIMULATION USING SPRAGUE-DAWLEY RATS (US ARMY AND NMRI/TD): Development of an animal model to simulate exposure encountered by Persian Gulf War veterans.
- Hereafter, Objectives, Accomplishments and Objectives for each workunit are referenced as A-E

*Smith, Zepp*

- Conduct blood gas studies with regard to homeostasis effects following serial blood collection/transfusions.
- Complete data books for SFE range-finding/multiple-dose, edema and blood gas studies.
- Submit revision back to journal editor for final consideration publication on SFE Formulation A pilot studies: part I and Part II.
- Conduct edema studies on SFE Formulation B, C and D.



## TECHNICAL OBJECTIVES FOR THIS REPORTING PERIOD

### *Abbas*

- Prepare a Protocol/Addendum to investigate the kidney tumor related glutathione pathway of TCE metabolism in rats.
- Continue data analysis and determination of metabolic rate constants for TCE metabolites.
- Perform sensitivity analysis on the PBPK model.
- Determine inter-conversion and percent yields of CH, TCOH, TCOG, TCA and DCA.
- Continue and complete the model development.
- Conduct more intensive literature review on TCE, its toxicity, pharmacokinetics, metabolism, and its metabolite toxicity and pharmacokinetics.
- Initiate and prepare a peer review PBPK model paper in mice for *Journal of Toxicology and Applied Pharmacology*.
- Continue to manage the TCE project, meet the customer needs, coordinate and oversee the TCE research work.

### *Ademujohn*

- Testing various pharmaceuticals on animal models using diminished capacity as the endpoint in Carneaux pigeons and Wistar rats.
- Range finding using operant-trained animals and measuring subsequent stages of diminished capacity.
- To compile, catalog and computerize the above mentioned data.
- To train pigeons and rats for problem solving protocols.
- Daily maintenance of pigeon intake and logging performance results.
- To obtain operant testing and training data for animals used in operant exposure
- To organize, catalog and generate computer graphics, cumulatively from the above mentioned data.
- To maintain data for future reference in upcoming publications.
- To be responsible for the procurement and securing of all materials used in testing and training protocols.
- Responsible for documenting and maintaining operant weights.
- Responsible for writing standard operating procedures for pigeon training protocols.
- Responsible for making daily accurate and detailed entries and updates of all work unit.



- Responsible for compiling information for conducting weekly meetings with/between work P.I.'s and laboratory technicians.

*Briggs*

- The primary objectives of this quarter were to continue to improve the efficiency and effectiveness of the toxicology research and assuring the quality and integrity of the data that was delivered to the study funding sponsors.
- Another primary function was to continue to improve the funding that supports the research functions at NMRI/TD and expand the resources for conducting human health risk assessments and develop toxicology characterization data for establishing appropriate exposure levels to environmental and occupational chemical hazards.
- A third objective of the quarter was to initiate the Quality Management Program and complete the Draft Standard Operating Procedures for use in conducting standard procedures for toxicology studies.
- The fourth objective was to review documents as tasked by the Officer In Charge to review strategic plans, veterinary services, Memorandum of Agreement with the Air Force and review research projects
- The fifth objective was to complete and submit the approved toxicology projects for Program 6 funding for FY97, and support Program 8 research projects to assure continuation of funding of current research and continuing to gain more funding for the future

*Carraci*

- Complete revisions and submit the Cardiac Sensitization animal use protocol to the Animal Care and Use Committee (ACUC) for approval.
- Coordinate scientific resources for Cardiac Sensitization project.
- Support efforts to continue inhalation experiments of Nonane for the TPH project.
- Complete data collection for direct probe method of determining blood:air partition coefficients



*Confer*

- Increase the population size of the *Hydra attenuata* colonies.
- Perform hydra assays and determine developmental hazard indexes (A/D ratios) on compounds to determine possible developmental toxicity.
- Assist in the preparation of a technical report on hydra assay.
- Prepare presentation and technical report on the metabolism of trichloroethylene by the medaka minnow.
- Support the Toxicology Division's Hazardous Materials/ Hazardous Waste Effort.
- Support the Good Laboratory Practices Process Action Team.

*Connolly*

- Catalog materials as received
- Catalog materials not yet cataloged
- Provide library service to the toxicology community at WPAFB
- Continue working on a manual card catalog

*Garrett*

- Support Dermal Penetration Project.
- Develop analytical methods to quantitate blood concentrations of chloropentafluorobenzene (CPF) in dermally exposed animals.
- Conduct *in vivo* dermal exposures of CPF in Hairless guinea pigs, Hartley guinea pigs and F344 rats.
- Develop research methods and analytical procedures to determine partition coefficients of perfluorohexyl iodide, CPF, and 1,2-dichlorobenzene (DCB) in skin of three test species.
- Coordinate jugular implantation surgery and dermal exposure experiments.
- Perform duties as Room Monitor.

*Geiss*

- Identify needs in molecular biology research support and design a technical approach to fulfill those needs.
- Develop protocols and research methods for the evaluation of biological effects of Air Force-related materials.



- Cooperate in current research relating to the toxicological effects of trichloroethylene (TCE), its metabolites and other compounds.
- Develop molecular methods for the Predictive Toxicology project.
- Train other scientists in molecular biology research methods.
- Participate in project planning for projects that include molecular biology methods.

*Grabau*

- Support Species Differences in Skin Penetration.
- Support Combustion Toxicology of Advanced Composite Materials (ACM) Project.
- Support 60 Day Study of Toxic Effects Trichloroethylene (TCE).
- Support Biologically-Based Dose-Response Modeling of Retinoic Acid.
- Support Preimplantation Effects of Ammonium Dinitramide (ADN).
- Support Program Development (Marketing).

*Horton, Rix*

- Completed installation of MS Internet Information Server
- Installation of MS Exchange Server (Enterprise) delayed due to late arrival of software
- Conversion of MS Mail and Post Office delayed due to late arrival of software
- Completed transfer and setup of 3 personnel to remote locations
- Ordered various software and hardware upgrades
- Uninstalled Windows 95 shell on Windows NT Servers
- Installed Windows 95 service pack 4 on all NT systems
- Upgraded memory in NT Servers
- Configuration of MS Browser and WINS for WAN delayed due to time constraints and reorganization of priorities
- Continued conversion of WFW systems to Windows 95
- Continued maintenance of Servers
- Continued backup of data files on Servers
- Continued support of hardware and software for TOXDET personnel
- Continued to update information Databases
- Continued to update all internet services (Web, Gopher, Listserv)



*Jung, Narayanan, T.K.*

The objectives for this period were to:

TMPP

Measurement of the amino acid and neurotransmitter concentrations in brain homogenate samples

Begin culturing the neuroblastoma cells for future use in experiments with TMPP

Cell Model

To obtain the metabolites of acetamidophenol for preparation of an HPLC standard curve

*Kimmel, Reboulet*

- Prepared and submitted proposal for Adult Respiratory Distress Syndrome (ARDS)
- Continued acquisition and implementation of combustion toxicology laboratory equipment.
- Continued toxicological evaluation of Spectrex Fire Extinguishant (SFE).
- Preparation of pulmonary physiology laboratory for Adult Respiratory Distress Syndrome study.
- Prepare scientific journal articles for review and publication.
- Prepared and presented airway model project for NAVAIR study.

*McDougal*

- Continue pharmacokinetic studies for in hairless guinea pigs with tridecafluoriodohexane and measure partition coefficients in the skin.
- Continue development of mathematical models for whole animal experiments.
- Measure diffusion of more MACS components across human skin.
- Estimate permeability constants for MACS components and provide information to customer.

*Narayanan*

- Evaluate neurotoxicity of quadricyclane in Sprague-Dawley rats.
- Detect and quantitate catecholamines and indoleamines in simulated PGW multiple chemicals' exposed male rats' serum.



- Measure and quantitate neurotransmitter levels in different regions of brains in control and simulated PGW chemicals' exposed male rats.
- Quantitate phosphoric acid in aerosol generated triaryl phosphate samples.

*Prues*

Technical support for the following NMRI/TD projects is to be provided:

- Spectrex fire extinguishant (SFE)
- Trimethylpropane phosphate (TMPP)
- Flow injection analysis (FIA)
- Serve as the Contract Representative on the Safety Policy Committee

*Ritchie*

- (A) To meet milestone objectives related to evaluation of 500+ rats on various NTAB tests during various pharmacological challenges, as described in a recently approved (2/96) animal use protocol. The overall purposes of this work unit is validation of several new NTAB tests, and predictive validation of the NTAB for human risk assessment. To complete development of juvenile play, audiogenic startle, Porsolt F.S.T., Morris Water Maze, Raw Hamburger Sniffing, Eyeblink Conditioning and Intracranial Self-Stimulation (ICSS) NTAB tests. To enact physical relocation of all neurobehavioral equipment at NMRI/TD to the WPAFB Veterinary Sciences Building.
- (B) To complete all neurobehavioral testing of ODS Halon-1301 using the Navy Roto-Wheel and two operant tests. To complete and submit a journal publication comparing the neurobehavioral toxicities of HFC-134a and CFC-12. To procure ODSRs HFC-236ea from DuPont and/or HFC-227ea from Great Lakes Chemical Corp.
- (C) To begin the physical construction, testing and validation of a totally computerized system to deliver to rats (during performance of NTAB tests) up to five mixed fire gases. Construction of the system was begun in February 96 in NMRI/TD Laboratories 202/203.
- (D) To begin scientific investigation of the effects of various human pharmaceutical drugs and anti-epileptic treatments on prevention of convulsive response to TMPP administration in rats. To assist with surgical techniques for implantation of cannulas and electrodes in rats for EEG/microdialysis or Flow Injection Analysis recording during direct microinfusion of TMPP and/or d-amphetamine into the caudate or substantia nigra.. To assist with surgical implantation of multi-unit stimulating and



recording electrodes in the nucleus accumbens and ventral tegmental area in rats. To continue investigation of the effects of single or repeated administration of very low doses of TMPP on development of long-term CNS sensitization in rats. To continue (at BGSU laboratory) a study to investigate the effects of chronic intrauterine exposure to low doses of TMPP on subsequent developmental toxicity and long-term CNS sensitization.

- (E) To complete all neurobehavioral evaluation of 64 or 128 rats used as subjects in the Persian Gulf War Simulation study.

*Smith, Zepp*

SFE Formulation A:

The objective of this research is to evaluate the potential health effects of exposure to the by-products of pyrolyzed SFE. SFE is fire suppressant and a potential replacement for Halon 1301.

Cardiac Sensitization:

The objective of this research is to develop an *in vitro* test for the determination of cardiac sensitization. These initial studies will set the basic background needed for future studies.

**SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD**

*Abbas*

TCE Project:

- Completed data analysis and determination of metabolic rate constants for TCE metabolites.
- Completed sensitivity analysis on the PBPK model.
- Completed inter-conversion and percent yields of CH, TCOH, TCOG, TCA and DCA.
- Completed the PBPK model development for TCE and P-450 metabolites in male mice.
- Completed more intensive literature review on TCE, its toxicity, pharmacokinetics, metabolism, and its metabolite toxicity and pharmacokinetics.
- Completed a draft of the peer review PBPK model paper in mice for *Journal of Toxicology and Applied Pharmacology*.





- Progressed on literature review to develop a Protocol to investigate the kidney tumor related glutathione pathway of TCE metabolism in rats.
- Continued to manage the TCE project, meet the customer needs, and coordinated the TCE research work.
- Worked on investigation of the role of gut microflora in the TCE metabolism, and conversion of TCA to DCA in the gut.
- Participated in TCE inhalation study.

Project Director:

- Submitted one FY97 execution plan, one quarterly and two monthly reports to SERDP, and two monthly and one quarterly report to the Director of Research Operation Council of the Toxicology Division.
- Updated the project folder according to the requirement of Director of the Research Operation Council to provide a central place for project information in the Toxicology division.
- Evaluated TCE project progress, milestone, man-hour, cost effects and established more effective timely progress plan to meet the customer's need.
- Contacted the scientists at USEPA, Harvard University, Wayne State University, Creighton University, and University of Wurzburg (Germany) regarding the results of our on going TCE research collaborations, and new research works.

Publications/Presentations, etc.

- Abbas, R., Seckel, C. S., Kidney, J. K., and Fisher, J. W. 1996. Pharmacokinetic Analysis of Chloral Hydrate and Its Metabolism in B6C3F1 Mice. *Drug Metabolism and Disposition*. In Press.

Ademujohn

- Compiled, organized, cataloged, via computer-aided graphics, the weekly data on Pigeon 'Match' and 'Shapes' protocols.
- Trained and conditioned new and incoming rodent and pigeon groups to protocol adaptation.
- Implemented an SOP on the procedure for pigeon "Match" protocol training.
- Implemented an SOP on pigeon injection protocol.
- Wrote procedures for Negative Pressure Acquisition in the Wahmann Chamber for pigeon conditioning.
- Maintenance of all laboratory work unit notebooks.



- Implemented several data methods to compile training data and weight maintenance on the pigeon operants.
- Cited as a contributor in the publications listed below:
- Trained summer students in all aspects of laboratory functions and procedures.
- Oversaw the collection and compilation and of the Neurobehavioral testing for the Persian Gulf War Study, such as photosensitivity, acoustic startle, and grip strength testing.

*Briggs*

- Assisted with the preparation and planning of research projects to be initiated in FY 97. Five new projects will be started which increases the Program 6 funding by more than 30% as compared to Fy96. The Adult Respiratory Distress Syndrome and Cardiac Sensitization projects will be managed by Geo-Centers toxicologists and will expand the spectrum of toxicology services at NMRI/TD
- Reviewed Draft Standard Operating Procedures and initiated planning for initiating the Quality Management Program next quarter
- Reviewed the NMRDC Strategic Plan and provided input as tasked by the Officer In Charge.
- Reviewed the Memorandum of Agreement between NMRI/TD and the Air Force. Represented the Navy in discussion with future Veterinary Services and provided recommendations as tasked by the O.I.C.
- Reviewed the DBNP research proposal and provided technical approach, milestones and costing data as tasked. This project will include developmental and reproductive toxicology studies which will be conducted and supervised by Dr. Briggs
- Prepared 16 Endocrine Disruptor inventory reports for the EPA sponsored workshop. These will be presented by CAPT Still who is the military representative on the Congressionally mandated committee that is assisting the EPA with establishing a test battery for assessing chemicals in the military environment for their ability to disrupt or mimic hormones that can interrupt the normal reproductive functions. Reviewed all 27 reports from the Tri-Service Toxicology Consortium and assisted in finalizing them for submission to the committee. This information will be used to promote the capabilities at WPAFB for performing reproductive system research.
- Attended two MCRA meetings with the EPA to perform risk assessments of chemicals of military interest and develop exposure levels. More than 20 risk



assessments were completed by the MCRA committee which met the SERDP funding objectives.

- Attended the Society of Teratology annual meeting and associated workshop to remain current with the new guidelines for reproductive risk assessment and animal research procedures.
- Assisted in the planning of contractor labor allocation to research projects during FY97 and interviewed candidates for the two expanded positions that have been funded. Offers to candidates are being extended and the new employees will be on board early in the next quarter
- Prepared a poster for the annual Command meeting which presented cardiac sensitization research
- Finalized the publication on reproduction policy for risk assessment

*Carraci*

- Halon 1301 Replacement Toxicity Project:
- Wrote the newly developed "Direct Probe Method" of determining blood:air partition coefficients as a peer-reviewed paper. As final data becomes available, paper will be finished and submitted for publication.
- Utilized the Direct Probe Method to determine rat and human blood:air partition coefficients for 1,1-Dichloroethane.
- Physiologically Based Pharmacokinetic Modeling of Cardiac Sensitizing Chemicals in Rats:
- Visited Dr. David Miletich and David J. Visintine, Michael Reese Hospital and Medical Center, Chicago, IL and learned proper techniques of surgically implanting an internal electrocardiogram (EKG) in rats, and learned how to estimate Epinephrine-induced ventricular arrhythmias.
- Wrote animal protocol and submitted to internal review committee for critique, and to the Animal Care and Use Committee for approval. Internal review committee included: Nancy Adams of the U.S. Environmental Protection Agency, Dr. Darol Dodd of ManTech Environmental Inc., Maj. Gary Jepson and Dr. John Frazier of the U.S. Air Force, and Dr. Jim McDougal of GEO-CENTERS, Inc.

Total Petroleum Hydrocarbon (TPH) Project:

- Completed series of Nonane nose-only inhalation experiments.
- Gavaged female Fischer-344 rats with Nonane/Soil mixtures. Exhaled air and blood samples were collected for Nonane analysis in order to develop a PBPK model.



- Alternate Pharmacokinetic (PK) Group Administrator:
- Coordinated resources, and time appropriation for 19 members of the PK group during Group Administrator's six week absence.
- Attended two Research Operations Council (ROC) meetings.

*Confer*

Defense Women's Health Research Program:

- Increased hydra population through careful maintenance and husbandry which resulted in the completion of multiple hydra assays.
- Co-authored technical report on hydra assay of Liquid Propellant XM46.
- Completed hydra assay of ammonium dinitramide.
- Completed hydra assay of ammonium perchlorate.

Trichloroethylene by the Japanese Medaka Minnow Project:

- Authored and presented poster at American Chemical Society Mid-West Regional Meeting.
- Co-authored government technical report.

Good Laboratory Practices Process Action Team:

- Served as GLP-PAT Secretary.
- Became more familiar with Toxic Substances Control Act; Good Laboratory Practice (GLP) Standards.
- Evaluated the areas selected to be GLP compliant, determined the feasibility of GLP and looked at what deficiencies exist that prevent GLP compliance within the Toxicology Division.
- Met with GLP consultants.
- Hazardous Materials/ Hazardous Waste Effort:
- Finalized Depot Maintenance- Hazardous Material Maintenance System (DM-HMMS) training for 27 room monitors.

Publications/Presentation, etc.

- Confer, PD, Buttler, GW and JC Lipscomb. Metabolism of Trichloroethylene by the Japanese Medaka Minnow. American Chemical Society Mid-West Regional Meeting, Dayton, OH. June 9-12, 1996.
- Lipscomb, JC, Garrett, CM and PD Confer. Chemical Metabolism as a Determinant of the Appropriateness of Test Species. Ecological Risk Assessment and Military Related Compounds: Current Research Needs Workshop. July 31 - August 2, 1996.



*Connolly*

- 37 books cataloged and prepared for circulation
- 29 articles obtained from local libraries
- 8 articles entered into the reprint database and file
- 6 interlibrary loans obtained
- 3 literature searches conducted using in-house CD-ROM database capabilities
- 3 searches successfully conducted on the internet for customers, including downloading of documents as required
- 12 reference questions answered
- 9 telephone inquiries on journal locations in local area handled successfully
- 39 requests for articles located and filled from in house resources
- 3 articles obtained using the CARL UnCover system via the internet
- 191 card sets prepared for manual card catalog
- 2 orientation trainings and 1 search methods training conducted
- 86 volumes received from bindery and processed in
- 34 volumes prepared for binding
- 198 journal volumes consulted by customers

*Garrett*

- Completed dermal penetration studies of perfluorohexyl iodide in three test species; F344 rat, Hairless Guinea Pig, and Hartley Guinea Pig.
- Continued partition coefficient determination of perfluorohexyl iodide in rat skin and began partition coefficient studies on guinea pig skin for both chemical:air and chemical:skin partitions.
- Developed analytical method for the determination of blood concentrations of CPFEB following dermal exposures in Hairless guinea pig.
- Began *in vivo* dermal exposures to CPFEB in three test species; Hairless guinea pig, Hartley guinea pig, and F344 rat.
- Coordinated jugular implantation surgeries and dermal exposure experiments for test animals.
- Supported Reference Dose for JP-4 project by performing jugular cannula implantation surgery in female F344 rats.
- Supported Trichloroethylene project by assisting with post-exposure organ harvesting in B6C3F<sub>1</sub> mice.



- Assisted in removal of hazardous materials from temporary storage site in building 79 into initial accumulation point for hazardous waste pickup.
- Attended conference for the AFOSR Dermal Focus Group held at Tri-Service Toxicology, WPAFB, August 6-7.

Publications/Presentations, etc.

- Lipscomb, JC, Buttler, GW and PD Confer. (1996). Trichloroethylene and Chloral Hydrate Metabolism in the Japanese Medaka Minnow (*Oryzias latipes*) *In Vitro*. AL/OE-TR-1996-0085. Armstrong Laboratory, Wright-Patterson Air Force Base, OH.
- Wolfe, RE, Kinkead, ER and PD Confer. (1996). Developmental Toxicity Screen of Liquid Propellant XM46 Using *Hydra attenuata*. AL/OE-TR-1996-(submitted for clearance). Armstrong Laboratory, Wright-Patterson Air Force Base, OH.
- J C Lipscomb, C M Garrett and P D Confer. Chemical Metabolism as a Determinant of the Appropriateness of Test Species. Abstract. Ecological Risk assessment and Military Related Compounds: Current Research Needs Workshop, July 31-August 2, 1996 Denver CO.
- J C Lipscomb, D Mahle, W T Brashear and C M Garrett. A Species Comparison of Chloral Hydrate Metabolism in Blood and Liver. *Biochem and Biophys Res Comm*. Submitted August 1996.
- J C Lipscomb, C M Garrett, and J E Snawder. Cytochrome P450 Dependent Metabolism of Trichloroethylene: Interindividual Differences in Humans. *Tox and Appl Pharm* Received 12 July 1996.
- J C Lipscomb, C M Garrett, and J E Snawder. A Quantitative Comparison of Cytochrome P450 Dependent Trichloroethylene Metabolism in Mouse, Rat and Human Hepatic Microsomes. *Tox and Appl Pharm* Received 12 July 1996.

*Geiss*

Group Administrator:

- Continued to assist in planning resource allocation, individual training and professional development for the PD group.
- Group mission and objectives are being integrated into the OET Strategic Plan in cooperation with the Laboratory management.
- Communicated with other scientists and project leaders to identify their technical needs and to suggest solutions.
- Time was spent on writing/reviewing/editing standard instructions for the lab on the preparation of Standard Operating Procedures (SOP's).



- Participated as a member of the Research Operations Council for OET.

TCE Support:

- Gene probes for target mRNA molecules were prepared and used for analysis of samples from the TCE study.
- Data obtained from the Northern analysis are being compiled for a peer reviewed publication, technical report and various poster presentations.
- Additional oligonucleotide probes were designed for synthesis. These are being used for Northern analysis and PCR.

AFOSR Support:

- The RNase Protection assay is undergoing method development.
- Attended American Society for Biochemistry and Molecular Biology Meeting, June 1-6, 1996.
- Attended 28th Central Regional Meeting, American Chemical Society, June 9-12, 1996.

Persian Gulf Project:

- Samples were obtained and stored for later molecular biology analysis.

Retinoic Acid Project:

- In situ hybridization analysis method developed for the analysis of histone mRNA in rat limb buds.

Confocal Microscopy:

- Oligonucleotides were prepared for later use in in situ hybridization analysis.

Laboratory Support:

- Time is being spent on authoring SOP's for molecular biology methods.

Publications/Presentations, etc.

- Lipscomb, JC, Buttler, GW and PD Confer. (1996). Trichloroethylene and Chloral Hydrate Metabolism in the Japanese Medaka Minnow (*Oryzias latipes*) *In Vitro*. AL/OE-TR-1996-0085. Armstrong Laboratory, Wright-Patterson Air Force Base, OH.
- Wolfe, RE, Kinkead, ER and PD Confer. (1996). Developmental Toxicity Screen of Liquid Propellant XM46 Using *Hydra attenuata*. AL/OE-TR-1996-(submitted for clearance). Armstrong Laboratory, Wright-Patterson Air Force Base, OH.



- J C Lipscomb, C M Garrett and P D Confer. Chemical Metabolism as a Determinant of the Appropriateness of Test Species. Abstract. Ecological Risk assessment and Military Related Compounds: Current Research Needs Workshop, July 31-August 2, 1996 Denver CO.
- J C Lipscomb, D Mahle, W T Brashear and C M Garrett. A Species Comparison of Chloral Hydrate Metabolism in Blood and Liver. *Biochem and Biophys Res Comm.* Submitted August 1996.
- J C Lipscomb, C M Garrett, and J E Snawder. Cytochrome P450 Dependent Metabolism of Trichloroethylene: Interindividual Differences in Humans. *Tox and Appl Pharm* Received 12 July 1996.
- J C Lipscomb, C M Garrett, and J E Snawder. A Quantitative Comparison of Cytochrome P450 Dependent Trichloroethylene Metabolism in Mouse, Rat and Human Hepatic Microsomes. *Tox and Appl Pharm* Received 12 July 1996.

#### *Grabau*

##### Support Species Differences in Skin Penetration:

- Methods were developed and utilized to quantify human Cutaneous endpoints in a manner similar to previous animal studies.
- Epidermal and dermal endpoints from animal in-vivo exposure were quantified.
- Drafting of a proposed publication detailing image analysis methods to quantify skin was begun.
- Attended 2 day meeting of the AFOSR Focus Group on Dermal Toxicology.
- Support Combustion Toxicology of Advanced Composite Materials (ACM) Project.
- Jointly authored a technical report currently under review titled: "Smoke Production and Thermal Decomposition Products from Advanced Composition Materials".
- Began development of ACM research results presentation for the 1997 Society of Toxicology meeting.

##### Support 60 Day Study of Toxic Effects Trichloroethylene (TCE):

- Completed quantification and analysis of hepatic ultrastructural peroxisome study.
- Jointly authored a paper for journal publication titled "A subchronic exposure to trichloroethylene causes lipid peroxidation and cell proliferation in male B6C3F1 mouse liver."
- Support Biologically-Based Dose-Response Modeling of Retinoic Acid:





- Developed methods to quantify bone deformities in fetal mice, trained and provided assistance for investigators in utilizing these methods.
- Provided consultation and developed a method to quantify zones of apoptosis in fetal limb buds.
- Began development of presentation describing methods to quantify bone deformities in fetal mice for the 1997 Society of Toxicology meeting.
- Support Preimplantation Effects of Ammonium Dinitramide (ADN):
- Developed a method to quantify three-dimensional size of nuclei in Preimplantation embryos. Performed and completed the analysis of the embryos.

*Horton, Rix*

- Orientated new employee (Mr. Horton) to system and procedures
- Identified and repaired I/O problem with main network server - reduced down time of system
- Improved scanner technology and repaired attached document feeder
- Developed initial draft for ADP SOP manual - while functional, it is still in development - this will assist NMRI to meet GALP guidelines
- Created wiring diagram for laboratory animal testing chambers to assist with stabilizing these chambers and to standardize procedures for diagnosing faults
- Received and implemented new version of virus protection software
- Improved Library server performance with 100% increase in RAM

*Jung, Narayanan, T.K.*

TMPP

- Rats were dosed with TMPP (0.5 mg/ kg) or Pentylenetetrazole (60 mg/ kg) and then decapitated immediately after tonic clonic severe grand mal seizures were seen. The brain was dissected into cerebellum, brain stem, frontal, middle, and cortical sections. These were frozen in liquid nitrogen and stored at -80 °C. A method was created and the standard curve measured for the detection of amino acids in the homogenates of these brain samples. The standards were prepared with o-phthaldialdehyde which binds to the amino acids and causes them to fluoresce. They were run on the HPLC in a mobile phase of 0.02 M Sodium Phosphate, pH 7.0, with methanol (25 - 50 %) and detected using a fluorescence detector. The homogenates were then run under isochratic conditions with Citric acid buffer (15 % v/v Methanol) for the detection of the concentration of neurotransmitters.



- The mouse neuroblastoma cell line was removed from cryostorage and put on plates in the incubator with media. The cell line will be cultured and cells periodically stored for future use.

Cell model

- Three rats were orally dosed with 0.2 g/ kg of acetamidophenol after being starved overnight. They were provided with food one hour after administration of the drug and with water at all times. Their urine was collected and pooled. The rats were dosed each day for four consecutive days and then allowed a break over the weekend. This was done for two weeks. The pooled urine was centrifuged at 10,000 rpm for 15 minutes. The supernatant was saved and lyophilized. The dry precipitate is stored at 4 °C.

Publications/Presentations, etc.

The four pre-proposals were written on:

- Molecular Approaches to Toxicity by Endocrine Disrupters
- Peroxisome Proliferation by Endocrine Disrupters
- Immunological Alterations by Endocrine Disrupters
- Cytochrome P450 Technology in Assessing the Safety and Efficacy of Endocrine Disrupters

SOT abstract is entitled "Effects of Trimethylolpropane Phosphate on Neurotransmitter Levels in the Rat Brain." A. Jung\*, T. K. Narayanan\*, and J. Rossi III. Naval Medical Research Institute (Toxicology Detachment) \*Geo-Centers, Inc., Wright-Patterson Air Force Base, Oh 45433-7903.

Paper entitled "The Effect of a Toxin on Cell Cultures" by D.P. Gaver, R.L. Carpenter, T. K. Narayanan, P.A. Jacobs, and A. Jung was submitted for approval for publication.

*Kimmel, Reboulet*

- Authored a paper entitled "Calculation Of Exposure Chamber Leak Rate With Thermal Corrections: A Measure Of Chamber Integrity And Performance " to be submitted for publication to the American Industrial Hygiene Journal.
- Authored a paper entitled "A Basic Computer Program for Rapid Standard Gas Bag Calculations" to be submitted for publication in Toxicology Methods.
- Specified and ordered a new Fume Hood for use in the pulmonary physiology laboratory.



- Initiated planning and renovations for ARDS laboratory
- Located and examined existing air handling capabilities, for pulmonary lab.
- Prepared Autocad (TM) drawings of the new pulmonary lab space for laboratory design criteria and coordinated plans
- Readied chamber, and support equipment for SFE exposures to be initiated next reporting period.
- Analyzed a program for probit analysis of Aerosols to determine their Mass Median
- Aerodynamic diameters, and Geometric Standard deviations. Discovered some questionable statistical methods in the program.
- Discussed statistics with statistician to determine analysis of data methods

*McDougal*

Air Force Office of Scientific Research (AFOSR) project (Dermal Penetration):

- Completed pharmacokinetic experiments with chloropentafluorobenzene in Fischer 344 rats and Hairless Guinea pigs.
- Determined skin partition coefficients with tridecafluoriodohexane and chloropentafluorobenzene.
- Hosted and presented at a meeting of the Air Force Office of Scientific Research Dermal Focus Group at WPAFB on 6-7 August.

Modular Artillery Charge System (MACS) project (Dermal Penetration):

- Completed development of analytical methodology for primary components of the propellant (nitroglycerin, diphenylamine, nitroguanidine, 4-aminobiphenyl, dibutylphthalate, nitrocellulose, and dinitrotoluene).
- Sampled propellant concentrations on surface of artillery charges in storage at Yuma Proving Ground.
- Provided analysis of the surface concentrations to the customer with interpretation.

Total Petroleum Hydrocarbon (TPH) project:

- Participated in pilot studies to determine the gastrointestinal absorption of nonane in soil in rats.

Other Scientific and Regulatory interactions:

- Continued to act as scientific mentor for Capt. Wade Weismann with emphasis on laboratory data collection.



- Invited to participate in a working group to recommend methodology for dermal reference concentrations to the US Environmental Protection Agency.
- Visited dermal research group, led by Capt Robert Casillias, at Edgewood Arsenal MD to discuss ways to collaborate on dermal damage projects in June.
- Participated in AFOSR sponsored meeting on Deicing compounds in jet fuels at Wright Laboratory on 5 August.
- Dr Barbara Hull and a graduate student from the Biology Department of Wright State University visited our laboratories to explore ways for collaboration. It was decided that the graduate student would do a lab rotation to learn dermal techniques and we would look for dissertation topics which might allow the student to do his Ph.D. research in our laboratory.

Publications/Presentations, etc.

- Jepson, G.W., and J.N. McDougal. Physiologically Based Modeling of Nonsteady State Dermal Absorption of Halogenated Methanes from an Aqueous Solution. *Toxicol. Appl. Pharmacol.*, submitted.
- McDougal, J.N. Prediction - Physiological Models. In Dermal Absorption and Toxicity Assessment. M.S. Roberts and K.A. Walters (EDS), Marcel Dekker, submitted.
- "In Vitro Dermal Absorption and Mathematical Modeling Tools for Dermal Exposure Assessment" " at Alternatives in the Assessment of Toxicity: Issues, Progress and Opportunities" Aberdeen Proving Ground, MD, 13 Jun 1996.

Narayanan

Acute, Subacute, Subchronic and Reproductive toxicity of Quadricyclane Vapor Project:

- Neurotransmitter levels were quantitated in different regions of rat brain to assess the neurotoxicity of Quadricyclane using HPLC.
- Reprocessed data for all the control and quadricyclane exposed samples.
- Statistically significant differences between control and quadricyclane exposed rats' neurotransmitter levels were estimated using student's t-test for unpaired data.
- Submitted technical report titled "Acute, Subchronic and Reproductive toxicity of Quadricyclane Vapor On Sprague-Dawley rats."

Toxicity Evaluation of Simulated Persian Gulf War (PGW) Exposure in Sprague-Dawley Rats, COET324:



- Submitted Standard Operating Procedure (S.O.P) for neurotransmitter analysis in different regions of rat brain using HPLC coupled with electrochemical detection.
- Control and PGW Chemical-exposed rat brains were surgically removed and nine regions of the brains were dissected and stored in freezer for future analysis.
- Eluting conditions for neurotransmitters were standardized using new C<sub>18</sub> column.
- Neurotransmitter analysis was performed in blood serum and different regions of rat's brain in control and PGW chemical-exposed rats.

Toxicity of Triaryl Phosphate Vapor Phase Lubricants:

- Eluting conditions for inorganic phosphate were standardized using anion exchange chromatography system.
- Phosphate in aerosol generated triarylphosphate samples was quantitated using linear regression of phosphate standard curve.
- Phosphoric acid was quantitated in aerosol generated samples using the acid conversion formula.

Other Duties:

- Literature survey was done to support future hormone studies.

Publications/Presentations, etc.

- Nicholas V. Reo, Latha Narayanan, Katrina B. Kling, Medhi Adinehzadeh (1995) Perfluorodecanoic acid, a peroxisome proliferator, activates phospholipase C, inhibits CTP:phosphocholine cytidyltransferase, and elevates diacylglycerol in rat liver. *Toxicology Letters* 86 (1996) 1-11.

*Prues*

SFE Project -- Homeostasis

- Equipment acquisition necessary to accomplish the cannulation procedure ourselves in-house.
- TMPP Project -- Glial Fibrillary Acidic Protein (GFAP)
- Acquired journal articles for a possible review article on GFAP

FIA Project-- Cyclic Voltametry

- Performed surgical implantation of combination electrodes being used for the in-vivo experimental portion of this project



- Assisted in the sensitivity evaluation of the combination probe being utilized in the experiment.
- Ran several test rats to refine experimental procedure and to test the system

*Ritchie*

- (A) The Tri-Service Neurobehavioral Toxicology Laboratory was totally and permanently relocated from NMRI/TD to the Veterinary Services Building. Five laboratory and animal housing spaces (2000 SF+) were dedicated to neurobehavioral research; two rat research laboratories, one pigeon research laboratory and two animal housing rooms. All assigned research is now being conducted at the VS facility. Nine pharmaceutical compounds were procured to commence NTAB validations research mandated by workunit .1605. Human, rabbit and rat eyeblink conditioned response equipment was developed; a new intracranial self-stimulation chamber system was manufactured at NMRI/TD and has been tested; a raw hamburger sniffing sensitization apparatus was built and tested; a tail flick system was purchased and tested; a forelimb grip strength testing system was tested; Morris Water Maze and Porsolt Forced Swim Test equipment were procured; a two-lane treadmill system was procured and tested; auditory startle response equipment (4 units) was assembled and tested; an Opto-Verimex (4 unit) locomotory response measurement system was rebuilt and updated; four figure-8 mazes were rebuilt and updated; EEG monitoring and audiogenic seizure measurement systems were rebuilt and tested. Twenty-one White Carneaux pigeons completed extensive spectral and pattern operant discrimination training in preparation for pharmacological challenges during performance of NTAB tests. Thirty (30) rats were implanted with electrodes in the nucleus accumbens or ventral tegmental area, and are currently being tested for intracranial self-stimulation response. Thirty rats are completing operant training for subsequent pharmacological challenge testing.
- (B) Workunit .1408 terminates 31 Sept '96. A close-out WUIS and NMRDC Final Progress Report were submitted. Twenty rats were tested for neurobehavioral toxicity from brief exposure to Halon-1301 as measured on the Navy Roto-Wheel; 8 rats were tested for cognitive/motivational deficits using an operant chamber. Proposed research for this quarter was not completed due to: (a) Great Lakes refusal to supply HFC-227ea to NMRI/TD for testing; (b) DuPont's refusal to supply HFC-236fa to NMRI/TD for testing; (c) the resignation of Inhalation Manager Larry Bowen. A journal article summarizing the majority of work completed was completed and is awaiting internal clearance.



- (C) Workunit .1420 terminates 31 Sept '96. A close-out WUIS and NMRDC Final Progress Report were submitted. Laboratory 203 of NMRI/TD was identified to house the new five-gas, whole body inhalation exposure system planned since 1993. Existing equipment in Laboratory 203 has been partially removed. Glenn Ritchie wrote the approved engineering plan for the laboratory. With the assistance of NMRI/TD, all required equipment for the laboratory was identified, ordered and received. The system will consist of: A Perkin-Elmer System 2000 FTIR for gas concentration monitoring; a Pentium computer system with artificial intelligence software for continuous control of chamber gas concentrations, 2 Matheson Gas Safety Cabinets (5 gas +2 gas) for housing of gas cylinders, computer-driven control valves, on-off solenoids, specialized regulators, and a gas mixing manifold; 2 sets of five industrial hygiene gas monitors for laboratory safety; an industrial chemical hood for system calibrations, four high capacity blower system for air input and exhaust control, and two modified Hinnert-type inhalation exposure chambers. The system, when completed, will allow exposure of laboratory animals to as many as five different gases during performance of NTAB tests.
- (D) Two new work unit proposals "Development of the Navy Neuro-Molecular Toxicity Assessment System (NTAS): Pharmacodynamic Modeling in the Development of a New Tool for Risk Assessment" (.1713; Rossi, Ritchie, Nordholm and Carpenter) and "Mechanisms Involved with Exposure to Select Neurotoxicants (.1712; Rossi, Ritchie, Nordholm and Aucker and Ahlers of NMRI) were written for FY97-2001 funding to continue work funded under workunit .1516 that terminates in 10/97. Three new summer Federal Research Interns were trained on a wide variety of neurobehavioral research techniques, including small animal surgery, and are currently performing a large percentage of the research related to this workunit. Thirty rats were implanted with EEG electrodes for testing with nine human pharmaceutical/anti-epileptic drugs to investigate prevention and treatment of convulsive response to TMPP administration. A paper (Narayanan, Jung, Ritchie and Rossi) was completed and submitted to Epilepsia describing the pharmacokinetic properties of TMPP. TMPP-induced CNS sensitization studies were completed using 20 rats; rats were electrically kindled and tested for sensitization as measured by audiogenic seizure susceptibility. A study involving EEG, microdialysis and behavioral analysis of 20 rats during direct microinfusion of TMPP and/or d-amphetamine into the caudate nucleus was begun. Rat neuroblastoma and human cortical cells are being plated for exposure to TMPP. A tissue slice system has been assembled.



- (E) 128 Sprague-Dawley rats were exposed for 6-hr per day for 14 consecutive days to (8) combinations of DEET, pyridostigmine, JP-8 vapors and/or unpredicted shock (stress) in THRU chambers. After a two-week rest period, 64 of the rats were housed at Veterinary Sciences for neurobehavioral evaluation. All rats were tested on: (1) Forelimb Grip Strength (12 trials); (2) Auditory Startle Response and Pre-Pulse Inhibition (80 trials); (3) Total Locomotory Behavior in 30 min (9 measures); (4) Raw Hamburger Sniffing (CNS Sensitization); (5) Analgesic Tail Flick Response (12 trials); (6) Two-Lane Treadmill Physical Fatigue (45 min); and (7) Photosensitivity (10 min). Rats were then sacrificed for blood, major organ and brain neurotransmitter (9 regions/5 neurotransmitters and metabolites) analyses.

*Smith, Zepp*

- Organization of data books for SFE range-finding/multiple-dose, edema and homeostasis studies. Several cardiac sensitization studies were initiated to study the mechanical and electrophysiological events leading to ventricular fibrillation.
- Submitted final drafts for technical reports on SFE Formulation A pilot studies: part I and Part II.

Start the following SFE protocols:

- Protocol NAV292: Evaluation of Blood Gas, Blood pH, Hemoglobin, Bicarbonate and Glucose Levels after Exposure to the Pyrolyzed By-products of SFE Formulation A
- Start studies on the development of an *in vitro* method for the determination of cardiac sensitization.

#### **GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD**

*Abbas*

- Submit the peer review PBPK model paper in mice for *Journal of Toxicology and Applied Pharmacology*.
- Conduct uncertainty analysis on the mice PBPK model.
- Complete development of the Protocol to investigate the kidney tumor related glutathione pathway of TCE metabolism in rats.
- Initiate and prepare a peer review pharmacokinetic paper for CH, TCOH, TCOG, TCA and DCA in mice.





- Support and participate in feasibility study on TCE non-cancer toxicity.
- Coordinate and submit (as a co-author) three analytical method papers for TCE, CH, TCA and DCA.
- Collaborate with Capt. Lipscomb on determination of effect of TCE exposure on P- 450 level in mice.
- Continue to manage the TCE project, meet the customer needs, coordinate and oversee the TCE research work.
- Participate in TCE pharmacokinetic Chapter preparation for USEPA.

*Ademujohn*

- To accurately and efficiently compile, log organize and analyze all incoming data from inhalation studies.
- To accurately train rodents for various testing protocols.
- To accurately train pigeons for upcoming testing protocols.
- To maintain a clean and orderly laboratory environment.
- To provide technical support in testing relative toxicity of various pharmaceuticals in pigeons and rats.
- To procure and document pigeon maintenance pertaining to preparatory requirements for 'shaping' activities, pre-testing and testing protocols.

*Briggs*

- Initiate the new projects and assure resources are in place to meet project objectives. This will require protocol preparation and approval and renovation of additional space in Buildings 839 and 824.
- Prepare pre-proposals for expanding the reproductive toxicology functions at NMRI/TD and institute the capabilities to support the research capabilities for the DBNP and other Program 8 projects
- Initiate the Quality Management Plan and review Standard Operating Procedures and finalize them for approval by management. Perform audits of critical phases and close out reports from research projects that end in FY96
- Attend Endocrine Disruptor, MCRA, EPA meetings on chemical risk assessment, and the Society of Risk Analysis meetings to remain current and solicit new business opportunities
- Complete the toxicology profile studies with the new ozone depleting substance replacement refrigerant and provide data to the Navy for establishing exposure levels



*Carraci*

- Complete the peer-reviewed paper on determining blood:air partition coefficients and submit for publications.
- Receive approval of Cardiac Sensitization animal use protocol and begin supporting research.
- Continue to support laboratory experiments for TPH project.

*Confer*

- Continue careful maintenance and husbandry of hydra colonies.
- Continue to perform hydra assays with additional test chemicals.
- Co-author and author additional technical reports on hydra assays.
- Submit abstract for 36th Annual Meeting of Society of Toxicology.
- Co-author paper on medaka for submission to Annals of Clinical and Laboratory Science.
- Assist the GLP-PAT in the preparation of a report for submission to Management on the feasibility of GLP within the Toxicology Division.
- Continue to support the Toxicology Division's Hazardous Materials/ Hazardous Waste Effort.

*Connolly*

- Continue cataloging
- Continue preparing cards for the manual card catalog
- Continue training program

*Garrett*

- Continue to provide support to Pharmacokinetics Group and Dermal Penetration Project.
- Complete dermal exposures of CPF in all three test species.
- Continue to coordinate and perform rodent surgeries for Dermal Penetration Project and other Pharmacokinetics Group projects as necessary.
- Begin dermal exposures of DCB in F344 rats, Hartley guinea pigs and, if possible, Hairless guinea pigs.



- Complete partition coefficient studies for perfluorohexyl iodide for all three test species and begin partitions with CPF and DCB.
- Write and submit abstract for Annual Society of Toxicology meeting before the Oct. 1 deadline.

*Geiss*

- Continue to develop molecular methods for use in our lab.
- Integrate new technologies into the battery of methods used to support Toxicology Division projects.
- Continue to perform assigned Group Administrator tasks.
- Continue to assist in training scientists in molecular methods.
- Continue to develop probes for use in hybridization experiments.
- Prepare a technical report on the Hyperbaric Oxygen project.

*Grabau*

Support Species Differences in Skin Penetration:

- Complete journal article for peer-reviewed publication.
- Quantitate epidermal and dermal endpoints following in-vivo exposures.
- Quantitate epidermal and dermal endpoints from human specimens.

Support Combustion Toxicology of Advanced Composite Materials (ACM) Project:

- Completed and submit jointly authored abstract for 1996 SOT meeting.
- Develop methods to correlate image analysis results from prior burns and newly developed apparatus.

Support Biologically-Based Dose-Response Modeling of Retinoic Acid:

- Completed and submit jointly authored abstract for 1996 SOT meeting.
- Continued support of current research efforts.

Support to Assessment of In-Vivo Biological Effects:

- Develop three-dimensional methods to quantify apoptosis from confocal microscope acquired images.

Support Program Development (Marketing):

- Continued support of the Program Development Strategic Plan.



*Horton, Rix*

- Continue developing ADP SOP manual
- Fully implement and successfully administer the new Navy RIMS software for tracking scientific proposals and tracking the status on various scientific projects performed at NMRI
- Develop comprehensive documentation for wiring, functions, and I/O for all laboratory testing chambers
- Develop and document a realistic and systematic mechanism for data archiving and preservation
- Update all internet services, to include new Web page for NMRI TOXDET, as well as optimizing Gopher and Listserv
- Initiate migration to new electronic mail software - Microsoft Exchange
- Initiate migration to new office suite software - Microsoft Office Professional
- Implement new CD ROM Library server system - this newly arrived hardware will greatly increase accessibility and speed for the comprehensive CD library for TOXLINE and MEDLINE
- Mr. Horton should attend the Microsoft Exchange Server Implementation conference
- Mr. Rix should attend the Lotus Notes conference

*Jung, Narayanan, T.K.*

- To increase the productivity in the lab
- To continue the TMPP binding studies
- To begin human cortical neurons for experiments with TMPP
- To continue the work with the rat liver cells of the cell model project
- To finish HPLC analysis of the neurotransmitter samples
- To culture and store neuroblastoma cells for experiments with TMPP
- To set up a method for the detection of the metabolites of acetamidophenol by HPLC

*Kimmel, Reboulet*

- Continue relocating the Pulmonary Physiology laboratory from Bldg. 433 to Bldg. 824
- Continue toxicological evaluation of Spectrex Fire Extinguishant (SFE).
- Submit technical papers and abstracts for presentation and publication.



*McDougal*

- Continue pharmacokinetic studies in hairless guinea pigs for chloropentafluorobenzene and 1,2-dichlorobenzene.
- Emphasize *in vitro* diffusion cells in the development of the mathematical models for the whole animal experiments.
- Complete organ weight data on Hartley guinea pigs and Fischer 344 rats for physiologically based model.
- Complete development of analytical methods for MACs *in vitro* exposures.

*Narayanan*

- Measure and quantitate neurotransmitters and their major metabolite levels in control and Triaryl Phosphate exposed rats using HPLC coupled with electrochemical detection.
- Measure and quantitate neurotoxic esterase and acetylcholine esterase enzyme levels in different regions of the rat brain in control and Triaryl Phosphate exposed rats.
- Support the Toxicity Evaluation Simulated Persian Gulf War (PGW) Exposure in Sprague-Dawley Rats project.
- Measure and quantitate neurotransmitters and their major metabolite levels in control and multiple chemical-exposed rats using HPLC coupled with electrochemical detection.
- Detect and quantitate Nonane in rat tissues exposed to different concentrations of Nonane by inhalation, using headspace GC, with on line Flame Ionization Detector (FID).
- Continue ongoing research projects by repeating some of the experiments and being involved in research projects that are of interest to Tri-Service.

*Prues*

- Completion of the Homeostasis portion of the SFE project.
- Incorporation of the GFAP immunoassay into the TMPP project for use in a proposed neurobehavioral toxicity model.
- Provide continued technical support for the FIA project



*Ritchie*

- (A) To continue NTAB predictive validation procedures by comparison of effects of pharmacological challenges (nine different drugs) on NTAB responses (seven tests) in rats and pigeons; to begin initial research related to enactment of the newly funded workunits "Development of the Navy Neuro-Molecular Toxicity Assessment System (NTAS): Pharmacodynamic Modeling in the Development of a New Tool for Risk Assessment" (.1713; Rossi, Ritchie, Nordholm and Carpenter) and "Mechanisms Involved with Exposure to Select Neurotoxins" (.1712; Rossi, Ritchie, Nordholm and Aucker and Ahlers of NMRI).
- (B) To write and submit the NMRDC Final Summary Report (5-20 pp) for Workunit .1409. To submit a scientific paper for journal publication.
- (C) To write and submit the NMRDC Final Summary Report (5-20 pp) for Workunit .1420.
- (D) To complete a major study evaluating potential therapeutic treatments (9 test compounds) for TMPP-induced toxicity; to support tissue culture studies for study of cellular-level TMPP-induced acute neurotoxicity; to assist in microdialysis/EEG/gross behavior studies investigating neurotransmitter consequences of systemic and localized TMPP and amphetamine administration; to support Dr. Eldon Smith in the development and start-up of neuroprotein (GFAP, vimentin, S-100, etc.) analyses related to TMPP-induced neurotoxicity; to complete and evaluate neurobehavioral consequences of repeated intrauterine exposure to TMPP in previously exposed juvenile rats; to support completion of studies related to CNS sensitization from repeated exposure to low doses of TMPP; to assist in start-up of CNS tissue slice studies to further evaluate the consequences of TMPP exposure on single unit responses and membrane characteristics; to develop three scientific posters related to TMPP neurotoxicity for the 1996 Society of Neurosciences meeting.
- (E) To test the remaining 64 exposed rats on each of seven neurobehavioral tests; to complete analysis of all neurobehavioral data from 128 rats; to prepare an initial scientific report for the Presidential Committee on Persian Gulf War Health Affairs.



*Smith, Zepp*

- Initiate pulmonary physiology studies on with regard to aerosol effects on the lung.
- Begin writing new protocols in the area of cardiac sensitization.
- Begin writing new protocols in the area of pulmonary toxicology.
- Begin developing GFAP assay for the neurobehavioral group to investigate subtle changes in the central nervous system.
- Complete protocol NAV292: Evaluation of Blood Gas, Blood pH, Hemoglobin, Bicarbonate and Glucose Levels after Exposure to the Pyrolyzed By-products of SFE Formulation A
- Close data books for SFE range-finding/multiple-dose, edema and blood gas studies.

**Note:** Karen Zepp resigned on 19 August 1996.



**V. NMRI, Natick, MA**

**A. U.S. NAVY CLOTHING AND TEXTILE FACILITY**

**DESCRIPTION OF WORK TO BE PERFORMED**

*Lacerte, Macek, Pawar, Schneider, Buller, Burke,  
Meyers, Monarrez, Donaldson, Collins, Madden,  
Grafton, Reynolds, Smith S., Smith/PM, Kubler*

**Program I: Flame Protective Clothing Research**

- The primary research goal for the current reporting period was to enhance essential fire testing capabilities of the Navy Clothing and Textile Research Facility (NCTRF).
- The fire tests related to the evaluation of thermal protective performance (TPP) of textiles involve large scale tests and bench scale tests. The large scale tests use the fire pit manikin under various types of thermal assaults including flames, radiation and heated steam. The bench scale tests are the TPP test and the Quartz Tube Heater test. Improvement of these test methods requires the identification of the test variables and establish their relationship to the test outcome. NCTRF needs to develop high precision instruments and techniques to identify these test variables and to study their influence on the TPP value of garment materials. Therefore, the goal was to develop a data acquisition (DAQ) utility and necessary sensor hardware. These tools will be used to enhance the existing tests or to assess the potential relevance of entirely different test methods.
- The next step was to actually develop the computerized TPP test. The computerization of the TPP test involved four main tasks:
  - Acquire voltage signal from the J Type thermocouple and convert it into scaled temperature values.
  - Plot the curve for temperature versus time.
  - Overlay the stroll curve on the above plot to predict the TPP rating of the given test fabric in terms of time to register a second-degree burn.
  - Offer the operator an easy to use graphic interface.
- The tools for test method development were evaluated from several vendors. National Instruments had the most appropriate instrumentation hardware and software for NCTRF's needs. A visit to National Instruments at Austin, TX was arranged by





the NCTRF, to attend a seminar on the LabWindows/CVI and to discuss the selection of the relevant developmental system.

Program II: U.S. Navy Certification Program for Commercial  
Environmental/Occupational (CEO) Protective Clothing/Equipment

- GEO-CENTERS, INC. will establish a program to be used by NCTRF to certify commercial off-the-shelf protective clothing/equipment as meeting or exceeding Navy functional performance requirements. This program will make possible the direct purchase of certified commercial protective clothing/equipment for shipboard use by Navy personnel.

Program III: Database Search

- Conduct an extensive search of databases to determine commercial, DoD and non-DoD government organizations with which the U.S. Navy Clothing & Textile Facility (NCTRF) may enter into cooperative R&D agreements for the research, development, and testing of dress and protective clothing systems.
- Determine cooperative opportunities for dual-use technology, technology transition, and technology exploitation.
- Prepare a technical briefing to highlight the technical expertise and unique facilities and equipment available at NCTRF. This briefing could be exploited by agencies seeking cooperative research, development, and acquisition agreements.
- Prepare documentation to convey the technical expertise and unique facilities and equipment available at NCTRF. This documentation could be exploited by agencies seeking cooperative research, development, and acquisition agreements.

Program IV: Great Lakes Prototype Footwear Test

- Provide technical support in the development of the Enhanced Chukka Shoe surveys for recruits, leaders, shipboard personnel, and Naval Academy personnel.
- Provide technical support for experimental design of study.
- Provide software support in the production of an on-line data entry program and database management.
- Provide data collection support at the Recruit Training Center (RTC).
- Analyze data by test group and write final report of findings of the study.



Program V: Technical Reports

- Analyze and organize information provided on projects conducted in the Navy Clothing and Textile Research Facility (NCTRF).
- Develop technical reports and articles for publication in peer-reviewed journals.

**TECHNICAL OBJECTIVES FOR THE REPORTING PERIOD**

*Lacerte, Macek, Pawar, Schneider, Buller, Burke,  
Meyers, Monarrez, Donaldson, Collins, Madden,  
Grafton, Reynolds, Smith S., Smith/PM, Kubler*

Program I: Flame Protective Clothing Research

Research goals related to automation of the TPP test were as below:

- Modification of DAQ functions for computerization of TPP Test.
- Development of mathematical solution for second-degree burn problem.
- Selection of developmental tools for the user interface.
- Design and development of the TPP test software itself.
- Debugging and testing of the TPP test software.

Program II: U.S. Navy Certification Program for Commercial Environmental/Occupational (CEO) Protective Clothing/Equipment

- Complete editorial changes to the certification program report and submit to NCTRF for review and comment.

Program III: Database Search

- A comprehensive modular briefing package will be prepared which will outline NCTRF's R&D programs, its capabilities, skills, facilities and unique test and evaluation equipment. This briefing package will be used by NCTRF management as it pursues its effort to form R&D alliances. In addition, other visual aids will be developed which can be used during briefings or as documents which can serve as introduction literature, prior to direct contact with potential alliance partners.



Program IV: Great Lakes Prototype Footwear Test

- Complete initial draft of technical report of study.
- Obtain comments and suggestions from all parties involved in study.
- Incorporate comments and suggestions into final technical report of the commercial Chukka Shoe wear test.

Program V: Technical Reports

- Analyze the data generated in testing a large variety of liquid cooling systems. The goal of the work is to provide design guidance to engineers in the selection of an appropriate liquid cooling system, and operating conditions for a particular heat load using graphic comparisons of performance and a correlative heat transfer equation.
- Continue to make editorial changes and incorporate comments from NCTRF into the following three reports:
  - "Development of a Rough Sea Simulation Method for Testing Protective Clothing"
  - "Validation of Rough Sea Simulation Methods for Testing Protective Clothing"
  - "Correlation of Thermal and Evaporative Resistances of Military Clothing Items, Measured on a Guarded Hot Plate and Thermal Manikin"

**SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING PERIOD**

*Lacerte, Macek, Pawar, Schneider, Buller, Burke,  
Meyers, Monarrez, Donaldson, Collins, Madden,  
Grafton, Reynolds, Smith S., Smith/PM, Kubler*

Program I: Flame Protective Clothing Research

- While computerizing the TPP test equipment, some important research tools were developed and some were discovered that facilitated the automation. The major achievements during this work are:
  - Development of thermal protection analysis (TPA) system
  - Acquisition of test development tools



- Configuration of computer hardware and the test equipment
- Demonstration of the ability to handle research project independently
- The major achievement in this quarter was the development of the thermal protection analysis (TPA) system in response to the NCTRF's proposal on automation of TPP test. The TPA system uses a slug copper calorimeter as a skin stimulant exposed to flame fires up to heat flux levels of about  $3.5 \text{ cal/cm}^2$ .
- Another important task achieved was the selection of the most appropriate software tool for automation of most of the laboratory test equipment at NCTRF. The C for Virtual Instruments called CVI from the National Instruments, was carefully evaluated and selected for developmental work. The graphic user interface for the TPP test was developed using these CVI tools. While the developmental tools were being procured, substitute C compilers and libraries were also procured. The TPA System was designed in a way to have the built-in flexibility for future modification into general purpose Data Acquisition (DAQ) utility.
- On the computer hardware side, the equipment from the National Instruments was configured to be used both as a portable multichannel and also single-channel system for data acquisition purposes. The wire mesh cloth for calibration of the TPP test sensor and heat source was also researched and procured. The response of the TPA system was validated by supplying known voltages to its input terminal. A more precise method of calibration is under development.
- For the ease of searching for a solution to the partial differential equations of heat transfer, a theoretical work involved in the formation of right boundary value problems (BVP) is in progress. The design of sensors and the holder assemblies depends on the definition of these BVPs.

Program II: U.S. Navy Certification Program for Commercial  
Environmental/Occupational (CEO) Protective Clothing/Equipment

- Finalized a draft with procedure and policies for the certification program of CEO protective clothing/equipment with emphasis on being compatible with current acquisition guidelines of using commercial performance procurement documents. This finalized version was submitted to NCTRF for review and comment.

Program III: Database Search

- GEO-CENTERS, INC. personnel scanned 120 photos into an electronic database. These scans were made from selected 35mm colored slides, 3x5 colored



transparencies, colored prints, 35mm negatives, brochures and catalogs. Copies of the scanned photos were furnished to NCTRF. An alphabetical index of all scanned material was prepared for ready reference.

- A briefing outline was prepared for review by NCTRF.
- Since existing photographs of the Traversing Instrumented Manikin could not clearly show this facility, two artists' concept drawings were prepared for use in the briefing.
- An initial draft of the briefing containing 72 vu-graphs was presented to the NCTRF for review. Based upon recommendations from NCTRF, the draft was revised and a revised version was submitted for review and comment.

#### Program IV: Great Lakes Protective Footwear Test

- The initial draft of the technical report was completed on 11 July 1996 and submitted to the Navy Clothing and Textile research Facility (NCTRF).
- Comments and suggestions were provided by NCTRF personnel and incorporated into the draft.
- A meeting of all Enhanced Chukka Shoe players was held on 10 September 1996. An enhanced draft report was presented for a final round of comments.
- Final comments were received from the NCTRF and the final technical report was completed and submitted.

#### Program V: Technical Reports

- Analyzed data generated from testing a large variety of liquid cooling systems. The tests were carried out on a thermal manikin with a sweating skin, measuring the power required to maintain the thermal manikin skin temperature at 95°F with cooling water at two inlet temperatures and three flow rates.
- Measurements were conducted in duplicate for two inlet temperatures and three flow rates, providing twelve individual measurements. The modeling approach involved averaging the results for the nominally duplicated conditions, to obtain six independent entries. A MathCad search procedure was used to determine the six unknown coefficients in the empirical heat transfer equation. In plotting the data from an early cycle of testing, it was found that there were problems in the trend of the data. A later upgrade of the sweating skin on the thermal manikin and recalibration of the flow meters provided nine sets of data which appeared to be well behaved when plotted. The use of a MathCad matrix inversion method was found to



be the more efficient method of solution. Although it was possible to generate coefficients for all nine sets of data, there were problems with the magnitude, sign and stability of the coefficients. Using Polymath software, acquired from the Chemical Engineering Dept., Tufts University, multiple linear regression least squares determinations of the coefficients were run using all twelve data. This method also provided confidence limits on the coefficients, which were proved to be very large, as a result of the small numbers of data for the large number of unknowns.

- Empirical two parameter relations were also developed which provided improved confidence limits on the unknown coefficient but at the cost of a poorer fit to the data. In consulting with two statisticians at Natick, it was suggested that the prediction might still be satisfactory with the original six coefficient model although there was no way to obtain a useful assessment of the accuracy. As a result of a recent discussion with the project officer, it was decided to accept the limitations of the approach which was judged to be useful for interpolation within the range of the temperature and flow rates used.
- Continued work on completing three separate reports. The two reports involve the development of a laboratory method for simulating rough sea conditions in the Navy Clothing and Textile Research Facility hydro-environmental tank. Both draft reports were reviewed by NCTRF and then circulated to additional reviewers. Comments have been incorporated into the report entitled, "Development of a Rough Sea Simulation Method for Testing Protective Clothing."
- The second report "Validation of Rough Sea Simulation Methods for Testing Protective Clothing" was completed as a final draft and submitted for review by the Navy.
- The third report, "Correlation of Thermal and Evaporative Resistances of Military Clothing Items, Measured on a Guarded Hot Plate and Thermal Manikin" was delivered to the NCTRF project officer for review.

#### GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

*Lacerte, Macek, Pawar, Schneider, Buller, Burke,  
Meyers, Monarrez, Donaldson, Collins, Madden,  
Grafton, Reynolds, Smith S., Smith/PM, Kubler*

Program I: Flame Protective Clothing Research



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- Selection of data acquisition system and sensor for the steam manikin
- Selection of skin simulant for sweating foot
- Evaluation of the response of the copper calorimeter at various heat fluxes on the automated TPP test equipment
- Evaluation of heat pipe thermal manikin and its controlled climate chamber for the acceptance criterion
- Development of manikin diagram for fire pit manikin test
- Search for the software developmental tools for the fire pit manikin

Program II: U.S. Navy Certification Program for Commercial  
Environmental/Occupational (CEO) Protective Clothing/Equipment

- Upon receiving comments from NCTRF on the certification program report, GEO-CENTERS, INC. will incorporate the changes into the report.

Program III: Database Search

- The modular briefing will be completed. A high quality color brochure, which outlines the NCTRF capabilities, product developments and specialized laboratories and equipment will be completed.

Program IV: Great Lakes Protective Footwear Test

- None

Program V: Technical Reports

- Complete the final editing and publication of the three pending technical reports.
- Begin a draft report on liquid cooling vests.
- NCTRF personnel had indicated earlier, interest in a published paper for a technical journal dealing with the rough sea simulation work, which would also include more recent results on this project. There has been no follow-up on this assignment.



ADDENDUM FOR QUARTERLY REPORT TWO

VI. NMRI, Natick, MA

A. U.S. NAVY CLOTHING AND TEXTILE FACILITY

DESCRIPTION OF WORK TO BE PERFORMED

*Lacerte, Macek, Pawar, Schneider, Buller, Burke,  
Meyers, Monarrez, Donaldson, Collins, Madden,  
Grafton, Reynolds, Smith S., Smith/PM, Kubler*

Program I: Flame Protective Clothing Research

Thermal Instrumented Manikin (ATIM) System Improvement

- The scope for the present manikin system requires improvement in two areas - the fire pit system itself, and the method for data acquisition and analysis. The TIM System evaluates thermal protective performance of fire protective garments. It reports predicted burn injury in terms of second-degree body burn. The short and long term goals for refining this system are discussed below.

Calibration of Heat Flux Transducer and Quartz Tube Heater

- The thermal hazard from radiant heat source is assessed on the NCTRF Quartz Lamp Tester. The heat source consists of five General Electric quartz tubes. The temperature of these tubes is normally measured using an optical pyrometer, however, this pyrometer is reported to be in a state beyond calibration and needs to be replaced. The work involves two major tasks - identification of the right kind of temperature measuring device and the development of instrumentation for heat flux measurement.

Installation and Calibration of Heat Pipe Thermal Manikin (HPTM)

- A new heat pipe thermal manikin is under development by a company located in Seattle. This manikin will be installed in the controlled environment chamber at NCTRF. In mid-April 1996, NCTRF and GEO-CENTERS, INC. Personnel will



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assess the manikin system in Seattle. The primary objective is to study the working of the manikin system, data acquisition hardware, and the driver software.

#### Analysis of R&D of Flame Retardant (FR) Degradation Indicator

- Currently, a project related to the development of FR degradation indicator is in its initial phase. NCTRF needed critical analysis of contractor's approach in developing an indicator that would turn on when the FR property of the garment was compromised.

#### Program III: Database Search

- Conduct an extensive search of databases to determine commercial and government organizations with which the U.S. Navy Clothing & Textile Facility may enter into cooperative R&D agreements for the research, development, and testing of life support equipment and protective clothing systems.
- Determine cooperative opportunities for dual-use technology, technology transition, technology transfer, and technology exploitation in the development of advanced protective clothing systems for shipboard use.

#### Program IV: Great Lakes Prototype Footwear Test

- Provide technical support in the development of enhanced protective footwear for shipboard personnel.
- Provide technical support for experimental design.
- Provide software support in the production of an on-line data entry program, and database management.
- Provide data collection support at the Recruit Training Center (RTC)
- Analyze data and write final report of findings.



## TECHNICAL OBJECTIVES FOR THE REPORTING PERIOD

*Lacerte, Macek, Pawar, Schneider, Buller, Burke,  
Meyers, Monarrez, Donaldson, Collins, Madden,  
Grafton, Reynolds, Smith S., Smith/PM, Kubler*

### Program I: Flame Protective Clothing Research

#### Fire Pit Manikin Test

- Analysis of current TIM System and the Medtherm heat flux transducer.
- Study of current method of second-degree burn calculation
- Enhance precision and confidence in burn report.
- Identification of areas for improvement.
- Present the detailed plan for innovation and methods to implement it.

#### Heat Flux Transducer and Quartz Tubes Heater

- Study of current temperature measurement on optical pyrometer.
- Study of radiant heat flux measurement techniques.
- Develop a sensor and its related instrumentation for radiant heat flux measurement for the quartz tubes.

#### Heat Pipe Manikin

- Visit the Measurement Technology North West Co. in Seattle to study and assess the operational capability of heat pipe thermal manikin and software.
- Degradation Indicator.
- Study various FR finishes for cellulosic fibers.
- Study the effect of laundry methods and chemicals on effectiveness of FR finishes.
- Design a theoretical model for an ideal FR degradation indicator.
- Assess proposed R&D method against facts obtained above.
- Present the results of critical analysis to NCTRF.



Program III: Database Search

- To access commercial, DoD, and other government databases to conduct searches for organizations which are responsible for the development of life support equipment and protective clothing.
- Determine the potential of engaging in cooperative programs which utilized the technical abilities, facilities, and special purpose equipment available within the U.S. Navy Clothing and Textile Facility.

Program IV: Great Lake Prototype Footwear Test

- Produce enhanced surveys for data collection effort.
- Meet with Navy representatives to produce experientnal design.
- Produce on-line data entry program and file formats for database.
- Travel to RTC to conduct initial surveys amongst recruits.

**SUMMARY OF WORK PERFORMED DURING CURRENT REPORTING  
PERIOD**

*Lacerte, Macek, Pawar, Schneider, Buller, Burke,  
Meyers, Monarrez, Donaldson, Collins, Madden,  
Grafton, Reynolds, Smith S., Smith/PM, Kubler*

Program I: Flame Protective Clothing Research

**Fire Pit Manikin Test**

- Obtained information about the current manikin system from NCTRF in a meeting on February 29, 1996. Studied the test reports and proposals on improvement of the current TIM System. Discussed current test protocols and test setup with NCTRF. Studied the 1996 work from Audet and et al, related to TIM System.



- Studied current sensor and data acquisition method. Developed plans for possible short-term and long-term improvements in sensor.
- Analyzed the current method of calculation of second-degree burn damage. Refined calculation an introduction of third-degree burn damage into current TIM System.
- Presented the proposed improvements in TIM System at a meeting with NCTRF staff.

#### Heat Flux Transducer and Quartz Tube

- Critiqued the current method of temperature measurement using optical pyrometer and identified shortcomings.

#### Heat Pipe Thermal Manikin

- Obtained and installed copies of "Scenario" and "HSDA" models for heat stress analysis from NCTRF. Analyzing several other NCTRF research reports for using these models which are related to the heat pipe thermal manikin test evaluation.
- Obtained literature realated to heat stress decision analysis.
- Performed literature search for heat transfer models on heat pipe manikins.

#### FR Degradation Indicator

- Studied the statement of work related to FR degradation indicator tag.
- Analyzed the proposal from a contractor to execute development of such tag.
- Studied chemical and physical factors that could cause the loss of effectiveness of FR finish.
- Studied effects of laundry methods and detergents on FR finish.
- Summarized and presented the critical analysis of R&D involved in development of FR degradation indicator to NCTRF.

#### Program III: Database Search

- Comprehensive searches were completed utilizing three separate databases. The Carroll Publishing database for commercial and U.S. government were used to identify organizations which are engaged in the development of life support equipment and protective clothing. A complete search was conducted utilizing the



Defense Technical Information Center's database on the DoD Research and Technology Work Unit Information System.

- Based upon the review of information and data obtained from these searches, a list of organizations which have the potential of becoming cooperative partners with the U.S. Navy Clothing and Textile Research Facility for research, development, and testing has been developed.

#### Program IV: Great Lakes Prototype Footwear

- The enhanced survey for recruits has been designed. The survey incorporated some questions from previous Navy surveys but others were introduced, including questions with rating scales. The questionnaire was designed with language to be understood by the new recruits. The survey included a demographic, and Turn-in and Re-issue supplements.
- The design of the "leader" part of the study was finalized. Leaders will be randomly assigned to one of two groups. One group will wear one pair of boots for three weeks, and then switch to their other brand, and vice versa. The leaders will be given the same questionnaire as the recruits after they have worn each pair of boots. After six weeks, the leaders will also complete a boot-comparison questionnaire which has also been completed.
- The shipboard personnel questionnaire was designed. It is similar to the recruit's survey, however certain questions have been modified to gain information specific to ships.
- The on-line data entry program has been produced. The program presents each of the questionnaires through a graphical user interface. The program automatically checks the input to make sure that it is valid. The data files output by the system are database format compatible.



## GOALS/OBJECTIVES FOR NEXT REPORTING PERIOD

*Lacerte, Macek, Pawar, Schneider, Buller, Burke,  
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### Program I: Flame Protective Clothing Research

#### Fire Pit Manikin Test

- Computerize bench scale TPP test.
- Develop various TPP sensors and the sensor holder hardware.
- Select and install data acquisition hardware, software and the computer for instrumentation.
- Develop computer interface for data acquisition.

#### Heat Flux Transducer and Quartz Tube

- Develop a sensor and instrumentation for radiant heat flux measurement for m quartz tubes.
- Test and validate the test method by using materials with known results.

#### Heat Pipe Manikin

- Installation of manikin in NCTRF lab and debugging of hardware and software.
- Calibration of the manikin system to verify and validate its test results using standard garments.

### Program III: Database Search

- An implementation plan will be prepared and delivered which will outline potential partners and follow-up activities which the U.S. Navy Clothing and Textile Facility can pursue to achieve its Strategic Plan. This plan will allow NCTRF to enter into cooperative agreements which will exploit dual-use technology, technology transition, technology transfer and leverage its technical abilities, facilities, and equipment.



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Program IV: Great Lakes Prototype Footwear Test

- Construct database for all data.
- Travel to the RTC and administer the surveys for two more data collections
- Enter RTC data from the leaders, shipboard personnel and Naval Academy personnel, into study database.
- Statistically analyze data using accepted techniques.
- Write final report of the findings of the study.



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